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NEW DEVELOPMENTS IN CEREAL CHEMICAL RESEARCH

CLINTON L. BROOKE

Pillsbury Flour Mills Company, Minneapolis, Minnesota

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The purpose of this paper is to summarize recent progress in cereal chemical research as reported in journals not generally available to the American cereal chemist. For this reason, papers published in CEREAL CHEMISTRY have with a few exceptions been omitted, since most cereal workers subscribe to this journal and read it carefully and regularly.

"Chemical Abstracts" covers the most important foreign literature, but many cereal chemists neither subscribe to this publication nor do they have ready access to its files. The little German journal "Das Mühlenlaboratorium" has an excellent abstract section, but is not widely circulated in this country.

There has been no lack of reviews of cereal chemical progress during the last few years. I need only mention Dr. Bailey's excellent review published last fall (1934); also those of Fairbrother (1932, 1934) and Herd (1933) in England, Geddes (1934) in Canada, and Masling (1932) in Germany.

It is of course impossible, even in the generously long period allotted this paper, to review all the papers published during the last year (June, 1934 to June, 1935). In certain important fields much work has been done but nothing new has developed, while in others, such as the study of flour strength and the nature of the changes occurring in flour during storage, new ideas and methods have appeared which may prove to be of great value.

It was something of a surprise to find upon surveying the literature that there were over 100 papers more or less worthy of mention in this review. From these have been selected 75 which may be of interest to the American cereal chemist. Crop and varietal surveys, studies of established methods and the like have for the most part been omitted, also special subjects reviewed in other papers on the Convention program.

Flour Strength

The so-called Göttingen method of testing wheat quality, which may be considered an elaboration of the dough-ball method originated by Saunders and elaborated and modified by Pelshenke as well as by Cutler and Worzella, has been described in several papers by Engelke (1934, 1934a, 1934b, 1934c, 1934d). According to reports, this method is finding considerable favor in Germany, and in some quarters is considered superior to the dough-ball test.

Apparently the method is essentially the same as that of Winter and Gustafson (1934), although more elaborate. A dough-ball made with wheat meal or flour, water, yeast and salt is immersed in a fermentation vessel provided with an overflow tube and fermented until the dough-ball disintegrates. Water displaced from the vessel by the expansion of the dough drips into a graduate placed on a scale provided with an automatic curve-drawing device. The curve shows gas production and retention as well as the specific gravity and stability of the dough. Proteolytic activity can be determined from the gas-retention figures for a series of doughs fermented for varying times before testing. Approximate flour yield is deduced from the ratio between the gas-retention figures for flour and for wheat meal, and baking quality and loaf volume can be forecast from the gas-retention data. By another modification the specific gravity of the grain can be determined. The amount of wheat-meal or flour used may be as little as 10 grams.

By applying the methods and apparatus used in the testing of metals, two English investigators, Schofield and Scott Blair (1932–33, 1934) have demonstrated that dough has mechanical properties similar to those of metals, such as work hardening, elastic after effect and hysteresis. While admitting freely that much work remains to be done before their methods can be put into practical application in the bread industry, they state that they consider it very likely that the behavior of the dough and the physical properties of the resulting bread are largely dependent on its viscosity, elastic modulus and relaxation time, and the way in which these properties are varied by physical treatment and fermentation. A special advantage of the methods used by these workers is that the results can be expressed in absolute units.

Berliner (1934) has given a formula for calculating the "theoretical baking number" of a flour from the percentage of gluten, the gluten swelling number, and the percentage of maltose. The constants in the formula must be adjusted to fit the baking procedure and numerical units employed. The formula is, however, applicable only to flours not treated with chemical improvers.

Support has been given to the theory that the formation of gluten is due to colloid-chemical phenomena, by the work of Reznichenko and Alakrinskaja (1935) at Moscow, U. S. S. R., who found that once the colloidal structure of the gluten proteins is destroyed, the original character of the gluten cannot be restored. Attempts to produce synthetic gluten by combining gliadin and glutenin in various proportions and in various manners were invariably unsuccessful.

Kozmin and Popzowa (1934), also at Moscow, examined a series of flours of varying gluten quality and found no relation between quality of gluten and the gliadin: glutenin ratio. They conclude that the differing qualities of the glutens from fresh flours may be due to hitherto unknown factors which influence the hydration capacity of the protein gel. Kozmin and Alakrinskaja (1935a) have observed the close relation between gluten quality and quantity and gas-retaining capacity of dough.

An application of peptization methods to the determination of flour strength is found in the work of Javillier and Djelatidès (1934), who determined the protein fractions soluble in 10% sodium chloride, neutral 70% ethyl alcohol, and 70% ethyl alcohol containing 0.3% of potassium hydroxide, and found that the mutual ratios of the different fractions, including the insoluble residue from the last extraction, were roughly correlated with the relative baking values of the flours studied. The ratios between the fractions soluble in 70% alcohol and in 70% alcohol containing 0.3% potassium hydroxide were the most significant, being 1–2 for the best flour, 2–3 for flour of indifferent or doubtful quality, and 3–4 for poor flours.

Rose and Cook (1935, 1935a) report that the viscosity of gluten dispersed in neutral solvents such as urea and sodium salicylate is higher than in alkali and acid, because its original properties are unchanged. Heat treatment of gluten dispersions indicated that gluten dispersed in neutral solvents, such as urea solution, is affected by heat in the same way as flour. Their work also shows that the difficulty experienced by various workers in obtaining consistent results in the determination of gluten quality by the technic of Sharp and Gortner may be due to the fact that the region of maximum viscosity is also the region of maximum instability.

Schulerud (1934) determined the viscosities of rye flour suspensions after 24 hours digestion at 30° C., and studied the effect of temperature, dilution with starch and increasing flour concentration on the suspension. Although no baking tests were made, the viscosity values permitted arranging the flours in the same order as assigned in practice on the basis of supposed baking value.

McCalla and Rose (1935) have carried out some very interesting and significant experiments on the fractionation of gluten dispersed in sodium salicylate solution, and found that although none of the successive fractions of gluten protein precipitated by magnesium sulfate are similar to gluten, a gluten can be obtained by redispersing, combining the fractions and reprecipitating the proteins as a whole. Gluten consists of a single protein complex which can be progressively fractionated and none of the results of the investigation lend any support to the classical view that gluten is composed of glutenin and gliadin.

A great deal of important work on the proteins has been done during the last three years by an English scientist, Dorothy Jordan Lloyd (1932, 1932a, 1933, 1933a, 1933b, 1934, 1935). Although her work has been closely linked up with the problems of the tanning industry, much of it has a more general significance. A number of American cereal workers are interested in the applications of her findings to the problems of cereal chemistry. An adequate summary of Miss Lloyd's work cannot even be attempted here—suffice it to say that she has thrown new light on the structure of the proteins, the relation of swelling to molecular organization, the pressure and water relations of proteins, the movement of water in living organisms and many other aspects of this complex subject.

Changes Occurring in Flour During Storage

Some of the most important contributions made during the period covered by this review have concerned the changes taking place during the aging or "ripening" of flour, with special reference to the role of the fatty acids. Most of this work has been done in Russia and Germany. Natalie Kozmin, working at the Russian Cereal Research Institute in Moscow, in collaboration with Katharina Alakrinskaja and Valerie Bondarev (1934), has demonstrated that the real cause of ripening of the gluten in stored flour is the hydrolysis of the fat through the accumulation of free unsaturated fatty acids. Removal of these acids from aged flour by ether extraction "rejuvenates" the flour by restoring the original character of the gluten, and when the acids thus removed are added to freshly-milled flour a marked aging effect is produced. The aging process can proceed even in the absence of oxygen, which is not essential to fat hydrolysis.

Reznichenko and Popzowa (1934) have supplemented Miss Kozmin's work with a more detailed investigation of the effect of various substances on the colloidal properties of gluten. After examining the effect of a large number of saturated and unsaturated compounds, as well as silver and mercury salts, formalin, and sulfuric acid, they

conclude that the active principle in the unsaturated compounds which exert a ripening effect on gluten is, first, their degree of unsaturation and, second, their inclusion in an homologous series, which may be described by the formula R − CH : CH − COO − M, where R is the radical or radicals and M a metal or hydrogen.

In connection with this work Miss Kozmin and Katharina Alakrinskaja (1935) have developed a method for determining the acidity of flour fat as an index of the age of flour. Their method is similar to the one described several years ago by Coleman (1929), but instead of extracting the greater portion of the fat with petroleum ether and titrating the fat in a solution of benzol and alcohol, they extract only as much of the fat as can be removed by 1 hour's boiling with benzol, dissolve the fat in alcohol and ether, and titrate with 0.01 N KOH. Fresh flour has an acidity number of 15–20 milligrams of 0.01 N KOH per gram of fat and the maximum attainable is about 180 milligrams. By this method aging can be followed and the point of optimum ripening can be determined with accuracy.

Miss Kozmin (1935) has also reported on her work, although in less detail, in CEREAL CHEMISTRY.

Kühl and Kliefoth (1934) in Germany claim that the keeping quality of flour compressed into cylinders under 300 atmospheres pressure is greatly enhanced—it will keep almost indefinitely, especially in an atmosphere of carbon dioxide. Kühl has also reported on his experiments in more popular manner (1934, 1934a), and has discussed proposals for improving the keeping quality of grain by drying and sterilizing.

Gömöry (1934) in Hungary also found that the fats of untreated flours decompose to free fatty acids on storing for over 6 months. The decomposition is accelerated in moist flours. In dried flours the unsaturation of the fats decreases, owing to oxidation.

Further storage experiments made by Kühl (1935) show that flours containing 0.5 to 0.75% of germ from which the fatty acids have been extracted, did not deteriorate in baking quality during 7 months' storage, while flours containing the same amounts of unextracted germ became rancid and bitter and fell off decidedly in baking quality. He attributes these changes to the effect of oxy-fatty acids formed by the action of oxidase on the free fatty acids naturally present in the germ. Whereas small amounts of the fatty acids of the unsaturated series improve the gluten quality of weak flours during storage, this is not the case with the oxy-fatty acids, which indicate the first stages of rancidity.

The effect of "peeling" on the keeping quality of grain and flour has also been observed by Kühl (1934b), who learned that dry peeling

effects practically complete sterilization if satisfactory provisions are made for removal of dust during peeling. Wet peeling merely increases and spreads bacterial infection.

Three French workers, Kling, Froidevaux and Dubois (1934), have employed the Chopin extensimeter to demonstrate that when fatty substances extracted from flour with cold petroleum ether were restored to the flour it showed the same extensimetric and analytical properties as before. The extensimetric pressure of the dough is greater the greater the ratio of gluten to fat. The accumulation of fatty acids in flour during aging increases the afflnity of the flour particles for water, especially water with a pH higher than 7, resulting in increased extensibility.

Moisture

The German milling journal "Die Mühle" has published a special number on moisture in cereals and cereal products, containing a number of valuable contributions. Fisher (1934), of the Research Association of British Flour Millers, reviews the principal methods used in determining moisture in flour, presents a statistical study of over 400 determinations of the effect of temperature and time on results obtained in the air-oven method, and makes comparisons between the air-oven method (3–12 hrs. at 110°) and a calcium carbide method which requires only 20 minutes and is less subject to error than the air-oven method. Müller (1934) describes a continuous electric moisture tester which records the moisture of grain moving into or within the mill, and gives equally reliable results with tempered and untempered wheat. The principle of this apparatus is explained in an article by Lippke (1934), who shows that electric moisture testers operating on the conductivity or dielectric capacity principles cannot give accurate results when the moisture is not uniformly distributed throughout the kernel, and claims that this source of error is eliminated in a new electrical tester in which the grain is introduced into a high frequency electrical field. The current induced in the grain is measured and recorded. Köster (1934) illustrates a forced-draft air oven with built-in scale, permitting weighing of the dried samples without removal from the oven.

A study of the errors involved in the air-oven moisture determination at 105° C. has been made by Pap (1934), who found that variations in the humidity of the air could cause a variation of $\pm 0.3\%$.

An useful review of the role of water in cereals and flour has been made by Kühl (1935a).

Many mill chemists will welcome two papers by Siepke (1934, 1935) on the fi!tration, clarification, softening, sterilization, etc., of

the water used for wheat washing, and on the purification and disposal of the waste water from the washers.

Ash

Müller and Köster (1934) have made a painstaking study of the various factors involved in the ash determination, inclduing preparation of sample, time of igniting porcelain crucibles, cooling time in desiccator, and temperature and time of ashing. Their work shows convincingly that the temperature of around 900° C. ordinarily employed in running fused ash is approximately the correct one, but the critical temperature range lies between 920 and 950° C. Within this range determinations can be made in 90 minutes with ordinary flours. Below 920° the fused slag is still viscous and may entrap particles of carbon, while above 950° the phosphates will decompose.

Bleaching Agents and Improvers

Roell (1934) in his book on improvers has provided a convenient guide to the principal organic and inorganic improvers and has also sketched the manufacture of malt flour and malt extract.

A survey of the principal flour bleaching and improving agents used in Europe, giving percentage composition, baking results and methods of detection and determination has been made by Lindberg (1934).

In connection with her work on the aging of flour, Miss Kozmin (1934) has studied the effect of chemical improvers on the properties of gluten, and through determining the strengthening effect on gluten of various oxidizing agents in relatively high concentrations, has reasoned by analogy that small doses, such as used with potassium bromate, work in the same direction, even though their effect is not directly demonstrable. Since her work was done with ether-extracted flours, she doubts the validity of the contention made by other authors that fats and lipoids are the immediate activators of the changes in gluten quality brought about by oxidizing agents.

That the normal curve of the farinograph—using salt and yeast—is not influenced by potassium bromate and ammonium persulfate is shown by Szegfy (1934). However, 10% flour suspensions containing persulfate were higher, and similar suspensions containing potassium bromate lower in water-soluble protein than untreated flour suspensions.

Potel (1934) believes that the mechanism of the changes in the plastic properties of dough under the influence of chemical agents is due to enzymic phenomena, and finds that the acceleration of the processes of oxidation increases the tenacity of the protein substances as measured by the Chopin extensimeter.

The Chopin instrument has also been used by Bruère and Courbe (1934) in determining the effect of biological improvers on the mechanical properties of dough. Soy bean flour and powdered milk produced an increase in tenacity (T) in standard flour and a higher equilibrium ratio between T and dough expansion.

According to Potel (1934), Demolon found that bromates, iodates, carbonates and benzoyl peroxide have no effect on the results obtained with the Chopin extensimeter, although a noticeable effect is produced by perborates, persulfates, sodium peroxide, potassium permanganate and hydrogen peroxide, even in very small amounts.

Unfavorable effects of improvers and bleaching agents on stored flour were noted by Gömöry (1934a), who found that after 6–18 months storage the quality of Hungarian flours treated with ammonium persulfate, benzoyl peroxide, chlorine or nitrogen trichloride was inferior to that of untreated flours.

Granulation

Gründer (1932, 1935) has followed up his work on the application of sedimentation analysis to the determination of particle size in flour, in a paper on the influence of size and free surface of the particle on gluten character and fermentation in wheat flour doughs. He describes studies made with the farinograph and fermentograph, in which it is clearly shown that the more gluten stability and elasticity improve with increasing size and decreasing free surface of particle, the less is the gas production and the longer the dough developing time. Since optimum fermenting power lies in the opposite direction from optimum gluten quality as far as granulation is concerned, Gründer recommends adjustment of these two factors by mixing coarse and fine flours in proper proportions.

Markley (1934), whose work is reported in CEREAL CHEMISTRY, has also studied sedimentation analysis as a means of determining flour particle size, using, like Gründer, a method based on the technic of Odén.

In another contribution Gründer (1934) presents ingenious flow sheets of a wheat and a rye mill, showing graphically the percentages of the total represented by all the products of milling, including the individual flour streams. Using the ash and yield figures he plots an "actual milling curve," from which the ash content of flour or feed of any given extraction can be read directly. By means of the "sink or swim" method of centrifuging finely-ground wheat and rye in liquids of varying specific gravity, fairly accurate and complete separations of the individual components of the grains were made. These, with further separations carried out on flour, have furnished material toward the construction of theoretical yield curves, which, although admittedly

inaccurate, are nevertheless in fairly good agreement with actual yield curves.

Studies of flour granulation made by Kranz (1934) bear out the findings of other authors as to the influence of granulation on diastatic activity and fermentation of dough.

Phosphorus Compounds

In a study of the phosphatides of wheat flour, Nottbohm and Mayer (1934) determined that 61–73% of the phosphatides is bound, not to the gluten complex, but to the starch, and that it is present in the form of lecithin. Practically all the phosphatide material can be extracted from flour by first mixing a given quantity with half its weight of water and an equal weight of pumice and then extracting several times with 1 + 1 alcohol-benzol mixture.

Channon and Foster (1934) have reported that the phosphatide fraction of wheat germ contains phosphatidic acid (as Ca, Mg and K salts), lecithin and cephalin, in the proportions of 4 : 4 : 1 when referred to the P content.

Determinations of the total phosphorus content of wheat and flour were made by Feyte (1933), who found that the phosphorus content of French wheats ranged from 0.80 to 1.41%, while flours ranged from 0.18 to 0.32%. No relation could be determined between phosphorus content and the Chopin extensimeter figure.

The lecithin content of wheat is discussed by Kühl (1935b), who attributes the superior quality of durum wheat for macaroni making to its high lecithin content. This may be the reason why Jesser (1934) found that satisfactory alimentary paste could be made from German *vulgare* wheat flours by the addition of lecithin.

Miscellaneous Methods and Apparatus

Using the dipping refractometer, Kuhlmann and Golossowa (1934) have been able to determine the concentration of the water-soluble solids in flour, dough and bread, and to follow the changes in concentration occurring during fermentation, baking and staling of bread. According to their hydrophyllic properties, flours may be arranged in the following descending order: Soy bean, rye, maize, hard wheat, soft wheat and potato flour.

A delicate test for rancidity in flour, semolina and alimentary pastes has been devised by Berlie (1934), consisting in extraction with a mixture of alcohol, ether and chloroform, addition of potassium iodide to the extract, digestion for 24 hrs. in the dark, and titration with dilute thiosulfate solution.

Karsten (1935, 1935a) reviews recent progress in the use of the

analytical quartz lamp and describes a new lamp employing solid electrodes of activated metal instead of mercury and an electrode vessel of dark glass which obviates the use of a special filter.

An unique spot test for estimating the amount of rye in wheat flour with the aid of the quartz lamp is given by Grosbüsch (1934). A drop of an alcoholic extract of the flour is placed on filter paper, allowed to dry, moistened with a drop of Fehling's solution, and examined under the lamp. The increasing fluorescence of the outer rim of the spot and the gradual change from blue to green indicate increasing proportion of rye flour.

We are indebted to W. W. Naschtschokin (1934), of the Scientific Research Institute for the Baking Industry at Moscow, for new data on the thermal conductivities and specific heats of flour, dough and bread at varying moisture contents and densities.

Müller (1934a) claims that the "Mahlautomat," a new laboratory mill employing two runs of conical stones instead of steel rolls, will give results very closely approximating those obtained on a modern commercial mill.

That the cereal chemist would play an important part in the event of war is pointed out by Seidel (1935), who outlines methods of protecting cereals and cereal products against warfare gases, and gives instructions for reclaiming products damaged by such gases. Seidel's paper has also been reported by Brooke (1935).

Thus we see that much important progress has been recorded during the past year. Cereal chemists throughout the world are constantly adding to our fund of knowledge, and each year's output establishes a new record of achievement. While no epoch-making discoveries have been made during the period covered, a great deal of hard work has been done, and many new and attractive fields of activity have been thrown open for further study.

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FURTHER STUDIES ON THE BAKING QUALITY OF FLOUR AS AFFECTED BY CERTAIN ENZYME ACTIONS

RELATIVE STARCH LIQUEFYING POWER OF THE DIASTATIC AGENTS INVESTIGATED

J. W. READ and L. W. HAAS

The W. E. Long Co., Chicago, Illinois

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In a recent paper (Read and Haas, 1934), dealing with the effects produced by certain enzyme products on bread doughs made from various flours which developed undesirable gluten characteristics, we presented data illustrating the beneficial influence exerted on the gluten by certain proteases during fermentation and baking. This publication also included a fairly extensive bibliography.

The present paper records the results of further studies with the proteases occurring in several diastatic agents and certain properties of the alpha and beta amylases prepared from these agents. Laboratory baking tests have been conducted with sponge doughs made from different flours which developed unfavorable properties, commonly referred to as "bucky."

Amylases and proteases are the important enzymes contained in certain commercial products which are purchased and utilized by the baking industry on the basis of their saccharogenic activity. Not until quite recently has the significance of proteolytic action in dough improvement received serious attention. On the contrary, it has been generally believed that the action of protease on gluten was actually more harmful than beneficial.

The protease factor contained in flour from malted grain and also in normal flour is believed to consist of a proteinase and a peptidase. The proteinase seems to be either a papainase or a closely related substance.

The amylase content of the common agents used in bread manufacture consists of two components, *viz.*, alpha-amylase (dextrinogenic) and beta-amylase (saccharongenic). The mechanism of the process by which the starch molecule is disintegrated and ultimately reduced to the alpha and beta forms of maltose respectively by the alpha and beta components of amylase remains undetermined.

Three different methods have been evolved for the separation of the alpha and beta amylases. That developed by Ohlsson (1926) and

Nordh and Ohlsson (1932) depends upon the destruction of the beta-amylase by heat and the inactivation of the alpha-amylase by acid. The beta-enzyme is essentially destroyed when heated at 70° C. for 15 minutes. The alpha-enzyme is inactivated at 0° C. at a pH of 3.3, obtained by the addition of tenth normal hydrochloric acid.

From their studies, Nordh and Ohlsson conclude that the amylase of ungerminated barley is pure beta-amylase; that alpha-amylase is formed only during germination, especially between the fifth and seventh days; that the amount of beta-amylase also steadily increases during germination, and the formation of both components is independent of each other, as is also their rate of action in disintegrating the starch molecule. They also pointed out that the characteristics of Taka-amylase and pancreatic amylase closely resemble those of the alpha-amylase of malt, and that the maltose producing power of alpha-amylase is small compared to that of beta-amylase.

The method devised by Van Klinkenberg (1932) utilizes precipitation by alcohol. Alpha-amylase is precipitated by 60% alcohol and beta-amylase is precipitated by 80% alcohol. By re-solution and re-precipitation of each component, also by taking advantage of the thermolability of the beta-enzyme, Van Klinkenberg was able to separate and prepare each amylase in a rather pure state in powder form.

Waldeschmidt-Leitz and Purr (1931) adjusted their enzyme containing extract to a pH suitable for the separate adsorption of each amylase on a special form of alumina, designated as "Cγ." Removal from the alumina was effected by washing with a citrate or a phosphate solution. They also isolated an activator from their malt extract, which they called amylo-kinase. This activator was likewise separately removed from their enzyme solution by means of the specially prepared alumina.

These investigators observed only beta-amylase in active form in ungerminated barley, but the addition of amylo-kinase to their alpha-amylase prepared from ungerminated barley enabled the alpha extraction to saccharify starch to a considerable degree. From this result they concluded that alpha-amylase is not completely absent in ungerminated barley, but is present in an inactive form.

In a more recent paper, Sherman, Caldwell, and Doebbeling (1934) have described a method whereby they succeeded in purifying malt amylase to a much higher degree than has heretofore been reported. They employed repeated fractional precipitation with ammonium sulfate, followed by dialysis, and a final precipitation with alcohol and ether.

Their product corresponded to the beta-component of malt amylase. At a dilution of 1 : 9,000,000 a typical preparation produced by this procedure formed approximately 10,000 times its weight of maltose from 2% starch in 30 minutes at 40° C. This corresponds to the most active preparations of pancreatic amylase so far obtained.

The amylase purified in this manner contained about 16% nitrogen, behaved like a typical protein in respect to precipitation and denaturation, showed the usual color reactions for protein, and lost its enzymic activity on denaturation. The freshly prepared material readily dissolved in water. In the process of purification, it was essential to maintain a temperature of about 0° C.

In connection with the studies herein presented, we have employed the Ohlsson procedure to separate the alpha and beta amylases. This method did not produce a quantitative separation, but appeared to be acceptable for our present purpose. It has been applied by Andrews and Bailey (1934) in their recent study of the amylases of normal and germinated wheat flours. In their extracts of germinated wheat treated according to Ohlsson's technique, they reported as follows concerning the percentage of activity possessed by their alpha and beta fractions prepared from germinated wheat as compared to the activity of their original extract:

	Acidified	Heated
	(Beta preparation)	(Alpha preparation)
Alpha-amylase	6%	80%
Beta-amylase	91%	12%

Experimental

The starch liquefying power of the diastatic agents employed in our studies was measured by the method described by Jozsa and Gore (1930). Briefly, this consists of the preparation of a standard starch paste prepared from the highest grade of potato starch (such as the B. K. M. F. grade, Joseph Morningstar and Company, New York City), and determination of the weight of starch liquefied when 100 g. of starch paste containing 4.211 g. of dry starch is acted on by 10 cc. of amylase solution for one hour at 21° C. The concentration of the amylase solution represents 0.1%, *i.e.*, 10 cc. represents the quantity of amylase extracted by water from 10 mg. of a given diastatic agent such as a malt flour.

A water-jacketed 100 cc. pipet, fulfilling certain specifications of the U. S. Bureau of Standards, was used to measure viscosity changes.

After one has acquired the technique of preparing, from day to day, a standard starch paste having a definite outflow time (within 5 to 10 secs.) determinations of liquefying power can then be made quite readily.

Jozsa and Johnston (1935) have very recently revised the method originally presented by Jozsa and Gore (loc. cit.) and described certain improvements in the experimental technique. Their standardization of the liquefying curve and preparation of a complete table of standard values materially simplifies the mathematical interpretation of results, and reduces to a minimum such errors as may be caused by the use of pipets having different outflow times.

Our results in connection with the liquefying power of the alpha and beta amylase fractions prepared by the procedure of Ohlsson are presented in Table II, and were evaluated in accordance with the standardized technique established by Jozsa and Johnston. In a companion paper, Johnston and Jozsa (1935) have presented a detailed description of the experimental studies involved in arriving at a general method for determining the concentration of enzyme preparations.

The method of calculation may be described briefly as follows:

From the outflow time of a given mixture, the percentage decline is calculated. By reference to this value in the proper column of the table, one may obtain the amount of starch liquefied. The activity or concentration of the enzyme preparation is then determined from the amount of liquefied starch by means of the empirical equation:

$$\text{Log}_{10} L = (S - 1078)\ (0.000565)$$

where L = liquefons per 10 ml. of infusion

and S = milligrams of starch liquefied in 1 hour

The number of liquefons per gram of preparation is determined from the concentration of the infusion and gives a measure of the enzyme content and also the liquefying power. The new type of enzyme unit termed the liquefon is defined "as that amount of starch liquefying enzyme which will convert the standard starch paste at the rate of 25 mg. of dry starch per minute at zero time under the specified conditions."

The precipitation of protease by safranine was carried out essentially according to the method employed by Tissue and Bailey (1931), who refer to Marston's earlier work (1923) with the azine and azonium compounds of proteolytic enzymes. In connection with their study of the proteolytic enzymes of malt preparations, Tissue and Bailey precipitated the protease contained in a water extract of the saccharifying agent (2% concentration), with an equal volume of a 0.5% solution of safranine.

In our baking tests, the azine precipitate was always used in water suspension. When so kept in the refrigerator, the proteolytic activity remained unimpaired for a considerable time. Precipitation with safranine, however, did not give a strictly quantitative separation of the protease. As a result, the diastatic filtrates from such preparations as Taka-diastase and Merck's diastase of malt (Medicinal-U.S.P. IX) still possessed greater proteolytic activity than did the highly diastatic malt flour H, Diamalt syrup (120° L.), or any other similar preparation which is utilized by the baking trade.

Compared to such products as Taka-diastase and diastase of malt (Medicinal-U.S.P. IX), the proteolytic power of the diastatic agents commonly used in bread manufacture is relatively small. This may be noted by the fact that the protease precipitated from 10 g. of malt flour H (see Fig. 7) caused no appreciable mellowing of the gluten when added to the sponge mix.

The relative proteolytic activity of the different products investigated was estimated by means of their liquefying action on gelatin. It is recognized that the gelatin test, as commonly used, is primarily qualitative. If it were available, a simple and rapid quantitative method for measuring proteolytic activity on gluten would serve a very useful purpose.

To enable the reader to gain a clearer concept of the experimental results obtained with the different flours, a number of photographic illustrations are presented to show the characteristic differences in crumb structure as produced by the different enzyme preparations. Volume values of the loaves photographed are also shown.

As stated above, sponge doughs were used in the baking tests. The sponge time was made 3½ hours instead of the customary 4½ hours. In connection with the flours investigated, it became evident that an additional hour produced no appreciable differences in the final results. The dough time was 20 minutes with an extra 25 minutes for the longer fermentation. The loaves were moulded by a commercial moulding machine. The following formula was used:

	Sponge	Dough
Flour	420 g.	280 g.
Water	Adequate	Sufficient
Yeast	10 g.	—
Arkady	3 "	—
Salt	—	12.25 g.
Sugar	—	28 "
Lard	—	14 "

The sponge mix was made with 60% of the flour, and received 60% of the total absorption plus 15 cc.

In all cases the enzyme products were dissolved in water as far as possible prior to their addition to the sponge. This practice was adopted to insure a uniform distribution of the enzyme throughout the sponge mix.

Two commercial enzymes and several products prepared in the laboratory were used in this work. The commercial enzyme products were as follows: diastase of malt (Merck-Medicinal-U.S.P. IX); Taka-diastase (Parke, Davis & Co.).

Malt flour H, and barley malt flour No. 3 were prepared in the laboratory. For the preparation of H, the wheat was allowed to germinate for 8 days at from 52° to 55° F. During this period, the acrospire had reached a length of around $\frac{3}{8}$ of an inch. Malt flour No. 3 was made from commercially malted barley known to have high diastatic activity.

The results of the numerous experiments are presented in tabular form in order to save space (see Tables I–VI).

TABLE I

RESULTS OF BAKING TESTS WITH SPONGE DOUGHS
(Scaling weight 500 g. Enzyme preparations added to sponge)

Enzyme product	Amount added	Loaf volume	Texture	Grain	Ovenspring
	Grams	*Cc.*	*Score*	*Score*	
Flour 133376—Saccharogenic activity 274					
Control	None	2320	94	93	Fair
Diastase of malt	0.35	2635	100	100	Good
Taka-diastase	0.30	2666	100	100	Good
Malt flour H	2.80	2265	92	90	Fair
Protease precipitate from 0.6 g. of diastase of malt (U. S. P. IX)		2650	100	100	Good
Protease precipitate from 0.6 g. of Taka-diastase		2684	100	100	Good
Protease precipitate from 2.0 g. malt flour H		2488	98	96	Fairly good
Protease precipitate from 0.8 g. of diastase of malt (U. S. P. IX)		2620	100	100	Good
Protease precipitate from 0.8 g. of Taka-diastase		2640	100	100	Good
Filtrate from 0.6 g. diastase of malt after protease precipitation		2496	98	96	Fairly good
Filtrate from 0.6 g. Taka-diastase after protease precipitation		2467	97	96	Fairly good
Filtrate from 0.8 g. diastase of malt after protease precipitation		2548	96	95	Fairly good
Filtrate from 0.8 g. Taka-diastase after protease precipitation		2425	96	95	Fairly good
Filtrate from 2.0 g. malt flour H after protease precipitation		2415	95	94	Fairly good

TABLE I—(*Continued*)

Enzyme product	Amount added	Loaf volume	Texture	Grain	Ovenspring
	Grams	*Cc.*	*Score*	*Score*	
Flour 129060—Saccharogenic activity 309					
Control	None	2245	92	92	Fair
Diastase of malt	0.350	2661	100	100	Good
Taka-diastase	0.300	2655	100	100	Good
Malt flour H	2.000	2200	92	92	Fair
Papain	0.021	2485	100	100	Fairly good
Pepsin	0.060	2548	100	100	Good
Protease precipitate from 0.5 g. diastase of malt (U. S. P. IX)		2600	100	100	Good
Protease precipitate from 0.4 g. Taka-diastase		2606	100	100	Good
Protease precipitate from 6.0 g. barley malt flour No. 3		2338	94	94	Fair
Protease precipitate from 28.0 g. diamalt (120° L.)		2450	96	96	Fairly good
Filtrate from 0.5 g. of diastase of malt after protease precipitation		2485	97	98	Fairly good
Filtrate from 0.4 g. of Taka-diastase after protease precipitation		2485	97	98	Fairly good
Flour 131489—Saccharogenic activity 280					
Control	None	2155	90	92	Poor
Diastase of malt	0.350	2636	100	100	Good
Taka-diastase	0.300	2640	100	100	Good
Malt flour H	2.800	2400	93	94	Fair
Barley malt flour No. 3	2.800	2395	94	94	Fair
Protease precipitate from 0.5 g. diastase of malt		2610	100	100	Good
Protease precipitate from 0.4 g. Taka-diastase		2650	100	100	Good
Protease precipitate from 4.0 g. malt flour H		2450	94	94	Fair
Flour 129901—Saccharogenic activity 295					
Control	None	2080	88	89	Poor
Diastase of malt	0.350	2512	100	100	Good
Taka-diastase	0.350	2500	100	100	Good
Protease precipitate from 1.0 g. diastase of malt (U. S. P. IX)		2534	100	100	Good
Protease precipitate from 1.0 g. Taka-diastase		2737	100	100	Good
Protease precipitate from 0.8 g. diastase of malt (U. S. P. IX)		2490	100	100	Good
Protease precipitate from 0.8 g. Taka-diastase		2646	100	100	Good

TABLE I—(*Continued*)

Enzyme product	Amount added	Loaf volume	Texture	Grain	Ovenspring
	Grams	*Cc.*	*Score*	*Score*	
Flour 129901—Saccharogenic activity 295					
Filtrate from 0.5 g. of diastase of malt after protease precipitation		2020	88	88	Poor
Filtrate from 0.5 g. Taka-diastase after protease precipitation		2072	88	88	Poor
Filtrate from 0.8 g. diastase of malt after protease precipitation		2320	93	95	Fair
Filtrate from 0.8 g. Taka-diastase after protease precipitation		2275	93	95	Fair
Filtrate from 1.0 g. diastase of malt after protease precipitation		2188	90	90	Poor
Filtrate from 1.0 g. Taka-diastase after protease precipitation		2190	90	90	Poor
Filtrate from 2.0 g. malt flour H after protease precipitation		2047	86	86	Poor
Flour 126587—Saccharogenic activity 208					
Control	None	1814	75	82	Very poor
Diastase of malt	0.350	2455	100	100	Good
Taka-diastase	0.300	2550	100	100	Good
Malt flour H	2.000	2065	82	85	Poor
Diamalt (120° L.)	7.000	2325	93	91	Fairly good
Protease precipitate from 0.6 g. diastase of malt (U. S. P. IX)		2400	99	99	Good
Protease precipitate from 0.6 g. Taka-diastase		2585	100	100	Good
Protease precipitate from 10.0 g. malt flour H		2150	85	89	Fair
Protease precipitate from 6 cc. yeast autolysate		2226	95	95	Fair
Yeast autolysate	6 cc.	2215	95	95	Fair
Yeast autolysate	8 cc.	2335	98	98	Fairly good
Flour 128457—Saccharogenic activity 293					
Control	None	1955	80	85	Poor
Diastase of malt	0.350	2400	100	100	Good
Taka-diastase	0.350	2450	100	100	Good
Malt flour H	2.800	1943	78	80	Poor
Protease precipitate from 0.8 g. of diastase of malt (U. S. P. IX)		2320	100	100	Fairly good
Protease precipitate from 0.8 g. of Taka-diastase		2468	100	100	Good
Filtrate from 0.8 g. of diastase of malt after protease precipitation		2200	90	90	Fair
Filtrate from 0.8 g. Taka-diastase after protease precipitation		2125	89	88	Poor

TABLE II

STARCH LIQUEFYING POWER OF VARIOUS ENZYME MATERIALS

Preparation	Liquefying period at 21° C.	Viscosity decline	Starch liquefied	Liquefons per 10 cc.	Liquefying power (66.7 × liquefons)
	Minutes	*%*	*Mgs.*		
Alpha-amylase from Taka-diastase[1]	60	50.3	802	0.698	47
Alpha-amylase from diastase of malt (Merck's U. S. P. IX)	60	74.5	1634	2.060	138
Alpha-amylase from malt flour H (wheat)	60	80.5	1932	3.036	202
Beta-amylase from Taka-diastase[1]	60	98.8	3698	—	937
Beta-amylase from diastase of malt (Merck's U. S. P. IX)	60	70.1	1443	1.610	107
Beta-amylase from malt flour H (wheat)	60	51.0	819	0.714	48
Aqueous extract of malt flour No. 3 (barley)	60	83.5	2103	3.793	253
Aqueous extract of diastase of malt (Merck's U. S. P. IX)	2	85.4	2220	4.419	294
	30	98.8	3698	—	937
	60	99.3	3910	—	964
Aqueous solution of Taka-diastase	2	79.0	1849	2.727	181
	30	99.2	3866	—	958
	60	99.5	3998	—	975
Aqueous extract of malt flour H (wheat)	2	67.7	1352	1.427	95
	30	93.3	2826	—	648
	60	96.8	3268	—	887
Diamalt (120° L.)	2	52.8	866	0.761	51
	30	85.9	2252		307
	60	91.4	2625		498
Filtrate from diastase of malt (Merck's U. S. P. IX) after protease precipitation	2	76.1	1706	2.264	151
	30	96.8	3268	—	887
	60	98.8	3698	—	937
Filtrate from Taka-diastase after protease precipitation	30	86.9	2319	5.037	335
	60	91.9	2697		548
Filtrate from malt flour H after protease precipitation	30	93.3	2826	—	648
	60	96.8	3268	—	887
Filtrate from malt flour No. 3 after protease precipitation	30	76.5	1727	2.325	155
	60	84.9	2189		283

[1] The amylase of Taka-diastase is an alpha-amylase which appears to be largely destroyed by heating at 70° C. for 15 minutes. It does not appear to be inactivated by hydrochloric acid at a pH of 3.3 at 0° C.

TABLE III

SACCHAROGENIC INDEX AND LINTNER VALUE[1]
SUBSTRATE

Diastatic agent	Flour No. 133376	Potato starch as purchased (B.K.M.F.)	Starch paste as prepared for liquefying power (B.K.M.F.)	Merck's soluble starch used ungelatinized (Lintner)	Merck's soluble starch (Lintner)
		Rumsey-Blish units			*Lintner units*
Diastase of malt	562	130	34,722	—	
Taka-diastase	503	90	23,106	—	
Malt flour H	478	60	28,975	—	
Aqueous extract of diastase of malt	515	—	33,364	350	224
Aqueous solution of Taka-diastase	506	—	23,104	345	175
Aqueous extract of malt flour H	375	—	26,695	315	200
Alpha-amylase from diastase of malt	106	—	—	70	
Alpha-amylase from Taka-diastase[2]	186	—	—	105	
Alpha-amylase from malt flour H	—	—	—	50	
Beta-amylase from diastase of malt	270	—	—	145	
Beta-amylase from Taka-diastase[2]	475	—	—	330	
Beta-amylase from malt flour H	—	—	—	75	
Aqueous extract of flour No. 133376	—	—	—	110	

[1] By the term "saccharogenic index" is meant the number of milligrams of anhydrous maltose yielded in excess of its own autolytic sugar production by 10 g. of the substrate when allowed to digest with 1% of the saccharifying agent for one hour at 30° C. The enormous differences in sugar produced from boiled starch (paste) and soluble starch are noteworthy.

[2] See footnote 1, Table II.

The baking test figures as shown in Table I represent in practically every case the summarized value of from three to four replicate bakes, including both the short and long fermentation periods.

The values for saccharogenic activity are, unless otherwise stated, expressed in Rumsey units and determined in a buffered suspension according to Blish's modification of the Rumsey method.

Discussion

The baking test data presented in Table I show the beneficial effects exerted on the gluten of "bucky" doughs by adequate dosages of protease obtained from diastatic agents having a high content of protease, such as commercial diastase of malt and Taka-diastase.

TABLE IV

SACCHAROGENIC POWER OF THE FLOURS AND DIASTATIC AGENTS INVESTIGATED

Flour No.	Saccharogenic activity of flour (autolytic)	Flour + 1% enzyme material digested 1 hr. at 30° C.					Ash [2]	Protein [2]
		Merck's diastase of malt (U.S.P. IX)	Taka-diastase	Malt flour H (wheat)	Malt flour No. 3 (barley)	Diamalt (120° L.)		
		Saccharogenic indices [1]						
133376	274	562	503	478	—	—	.433	12.44
133375	276	555	500	478	—	—	.429	12.45
131489	280	552	535	520	395	—	.453	12.74
129901	295	545	500	450	333	200	.400	11.87
126587	208	642	610	600	420	—	.371	12.46
129060	309	—	—	—	330	—	.400	12.28
128457	293	572	—	—	412	186	.394	12.83

[1] Saccharogenic index is the number of milligrams of anhydrous maltose yielded in excess of its autolytic sugar production by 10 g. of the flour when allowed to digest with 1% of the saccharogenic agent for one hour at 30° C.
[2] 15% moisture basis.

To what extent the gluten complex was modified by the protease is unknown, but the amount of proteolysis which occurred produced more lively and better working doughs, which yielded loaves of good volume, texture, and grain. The amylolytic activity of the several products investigated apparently played no beneficial role. Excessive dosages of amylase (as well as proteases) resulted in sticky doughs.

With flours that give rise to "bucky" doughs, it appears from the results obtained that the treatment required is to render the gluten more pliable within certain limits, by subjecting it to the action of plant protease, preferably that associated with saccharifying agents. The saccharogenic preparations now commonly used by the baking industry are relatively low in their protease content compared to certain commercial products, such as Taka-diastase and Merck's diastase of malt (Medicinal-U.S.P. IX). Tables V and VI give a qualitative estimation of the relative proteolytic power of the different diastatic preparations tested. The marked activity of Taka-diastase and diastase of malt will be noted.

In Tables III and IV, data are presented which show the relative saccharifying activity of the products investigated and in Table II their relative starch liquefying values are recorded. With reference to saccharifying power, our results justify the repetition of an opinion which has appeared a few times in recent literature, *viz.*, that the physical condition of the starch granule materially influences the quantity of maltose produced.

TABLE V

RELATIVE PROTEOLYTIC ACTIVITY OF DIASTATIC AGENTS[1]

Enzyme preparations were added to 20 cc. of 5% gelatine solution, containing a little thymol to prevent bacterial decomposition. Digestion period 24 hours at 25° C.

Enzyme solution added	Temperature	Consistency of gelatine after 24 hours digestion					
		5% extract of malt flour No. 3 (barley)	5% extract of malt flour H (wheat)	5% solution of Diamalt (120° L.)	2% extract of diastase of malt (U.S.P. IX)	2% solution of Taka-diastase	Gelatine control plus water
Cc.	° *C.*						
1	25	solid	solid	solid	liquid	liquid	solid
	2	"	"	"	"	"	"
2	25	solid	solid	solid	liquid	liquid	solid
	2	"	"	"	"	"	"
3	25	solid	solid	solid	liquid	liquid	solid
	2	"	"	"	"	"	"
4	25	solid	solid	solid	liquid	liquid	solid
	2	"	"	"	"	"	"
5	25	solid	solid	solid	liquid	liquid	solid
	2	"	"	"	"	"	"
6	25	solid	solid	solid	liquid	liquid	solid
	2	"	"	"	"	"	"
7	25	solid	sl. soft solid	solid	liquid	liquid	solid
	2	"		"	"	"	"
8	25	solid	soft solid	solid	liquid	liquid	solid
	2	"		"	"	"	"
9	25	solid	semi-solid solid	solid	liquid	liquid	solid
	2	"		"	"	"	"
10	25	sl. soft solid	semi-solid solid	solid	liquid	liquid	solid
	2			"	"	"	"

[1] Extracts of Taka-diastase and Merck's diastase of malt (Medicinal U.S.P. IX) appear to have, by virtue of the qualitative tests employed, from 40 to 80 times more proteolytic activity on gelatin than do the ordinary malt flours and syrups.

The data obtained on the starch liquefying power of the amylase components from malt and from Taka-diastase indicate that Taka-amylase is primarily a beta-amylase. This is contrary to the findings of Kuhn (1925), and is also contrary to our own results with diffusion tests on a starch gelatine medium as originally devised by Beyerinck (1889). It is now generally accepted that the amylase of Taka-diastase consists of the alpha-form and the discrepancies in connection

TABLE VI

RELATIVE PROTEOLYTIC ACTIVITY OF CERTAIN AMYLASE PREPARATIONS FROM TAKA DIASTASE AND MERCK'S DIASTASE OF MALT (MEDICINAL-U. S. P. IX)

Enzyme preparations were added to 20 cc. of a 5% gelatine solution which contained a little thymol to prevent bacterial decomposition. Digestion period 24 hours at 25° C.

Enzyme solution added	Temperature	Consistency of gelatine after 24 hours digestion					
		Alpha amylase from 1% solution of Taka-diastase[1]	Alpha amylase from 1% solution of malt diastase (U.S.P. IX)[1]	Beta amylase from 0.5% solution of malt diastase (U.S.P. IX)	Beta amylase from 0.5% solution of Taka-diastase	Filtrate from 2% extract of malt diastase after protease precipitation	Filtrate from 2% solution of Taka-diastase after protease precipitation
Cc.	°C.						
1	25	solid	solid	solid	solid	semi-fluid	semi-fluid
	2	"	"	"	"	semi-solid	semi-solid
2	25	solid	solid	solid	solid	semi-fluid	liquid
	2	"	"	"	"	semi-solid	"
3	25	solid	solid	solid	solid	liquid	liquid
	2	"	"	"	"	semi-fluid	"
4	25	solid	solid	semi-fluid	semi-fluid	liquid	liquid
	2	"	"	solid	solid	semi-fluid	"
5	25	solid	solid	liquid	liquid	liquid	liquid
	2	"	"	solid	solid	"	"
6	25	solid	solid	liquid	liquid	liquid	liquid
	2	"	"	solid	solid	"	"
7	25	solid	sl. soft	liquid	liquid	liquid	liquid
	2	"	solid	sl. soft	sl. soft	"	"
8	25	soft	soft	liquid	liquid	liquid	liquid
	2	solid	solid	soft	soft	"	"
9	25	soft	soft	liquid	liquid	liquid	liquid
	2	solid	solid	semi-solid	semi-solid	"	"
10	25	semi-solid	semi-solid	liquid	liquid	liquid	liquid
	2	solid	solid	semi-fluid	semi-fluid	"	"

[1] Heating for 15 minutes at 70° C. destroys most of the protease.

with starch liquefaction may be explained on the basis that the alpha-amylase of Taka-diastase is largely destroyed by heating at 70° C. for 15 minutes, and is not inactivated by hydrochloric acid at a pH of 3.3 at 0° C. as would be expected in accordance with Ohlsson's method of separating the alpha and beta components of malt diastase. Whether this difference in behavior is to be attributed to structural differences in the alpha-forms or to other factors remains unknown.

The photographic illustrations (Figs. 1–9) portray typical differences in crumb structure. They represent cross sections near the center of the loaf. Loaf volumes are also shown. Minor variations were made in focussing the camera with a view to emphasizing differences in crumb structure rather than volume variation.

Fig. 1. Baking results with flour No. 133376, showing effects on crumb structure and volume produced by diastase of malt, protease precipitate and corresponding filtrate from diastase of malt, when added separately to the sponge. In the dosage shown the filtrate contained, even after precipitation of protease, sufficient proteolytic power to give a reasonably good crumb structure in the bread from this flour.

Fig. 2. Baking results with flour No. 133376, showing effects on crumb structure and volume produced by malt flour H, protease precipitate from this flour and from Taka-diastase, and corresponding filtrates from malt flour H and from Taka- and malt-diastase, when added separately to the sponge.

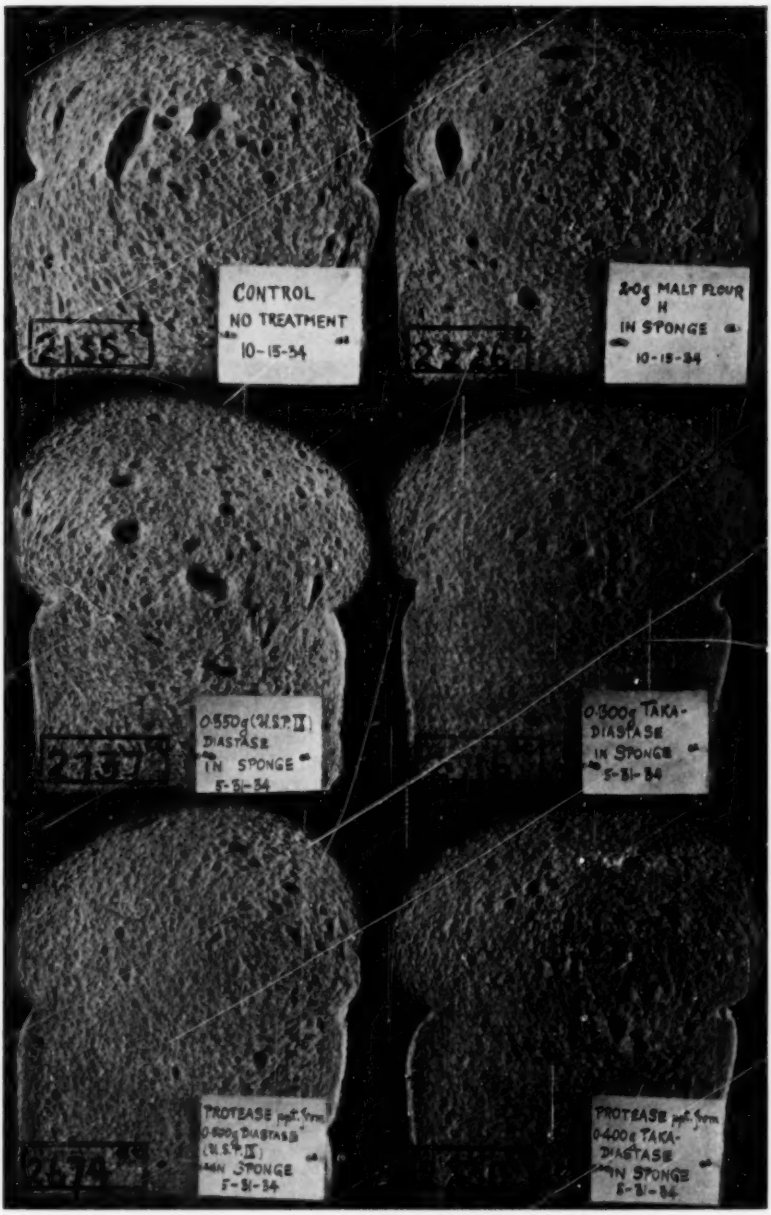

Fig. 3. Baking results with flour No. 129060, showing effects on crumb structure and volume produced by malt flour H, Taka-diastase, diastase of malt, and precipitated protease from Taka- and malt-diastase when added separately to the sponge.

Fig. 4. Baking results with flour No. 131489, showing effects on crumb structure and volume produced by diastase of malt, malt flour II, and by rather small dosages of protease precipitate from these and from Taka-diastase when added separately to the sponge.

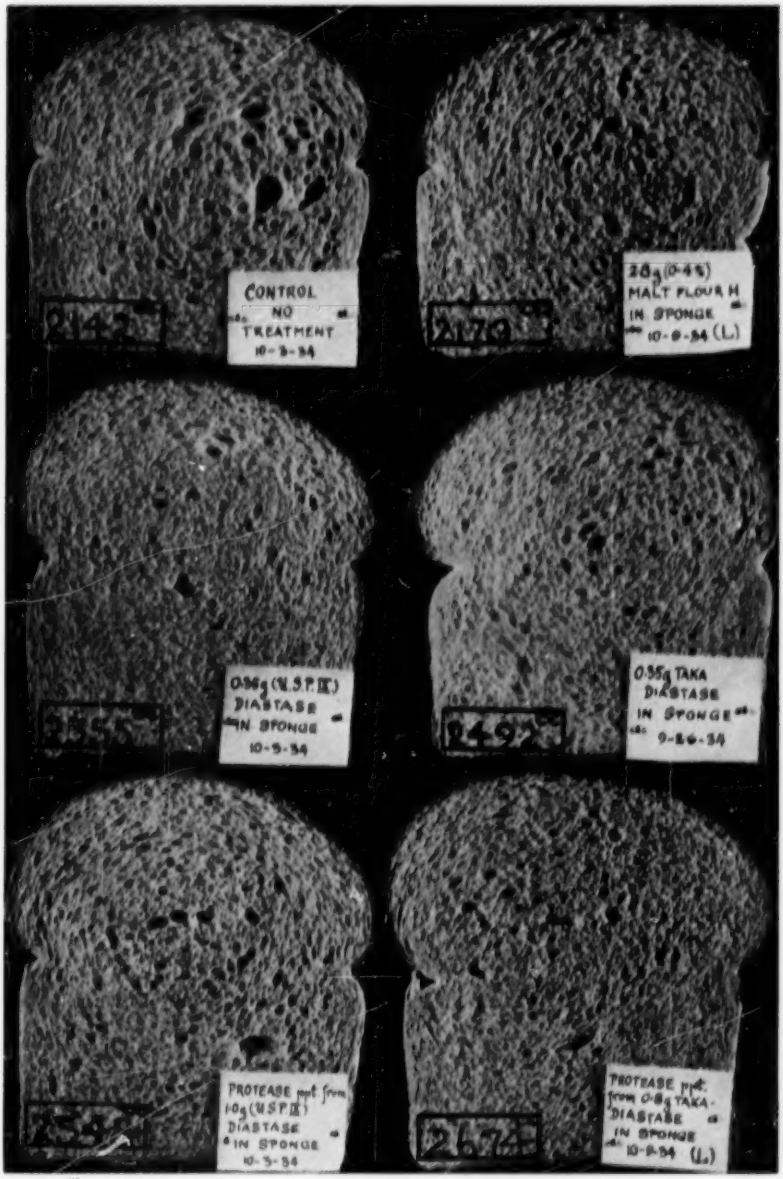

Fig. 5. Baking results with flour No. 129901, showing effects on crumb structure and volume produced by malt flour H, Taka-diastase, diastase of malt, and protease precipitate from the two latter products, when added separately to the sponge.

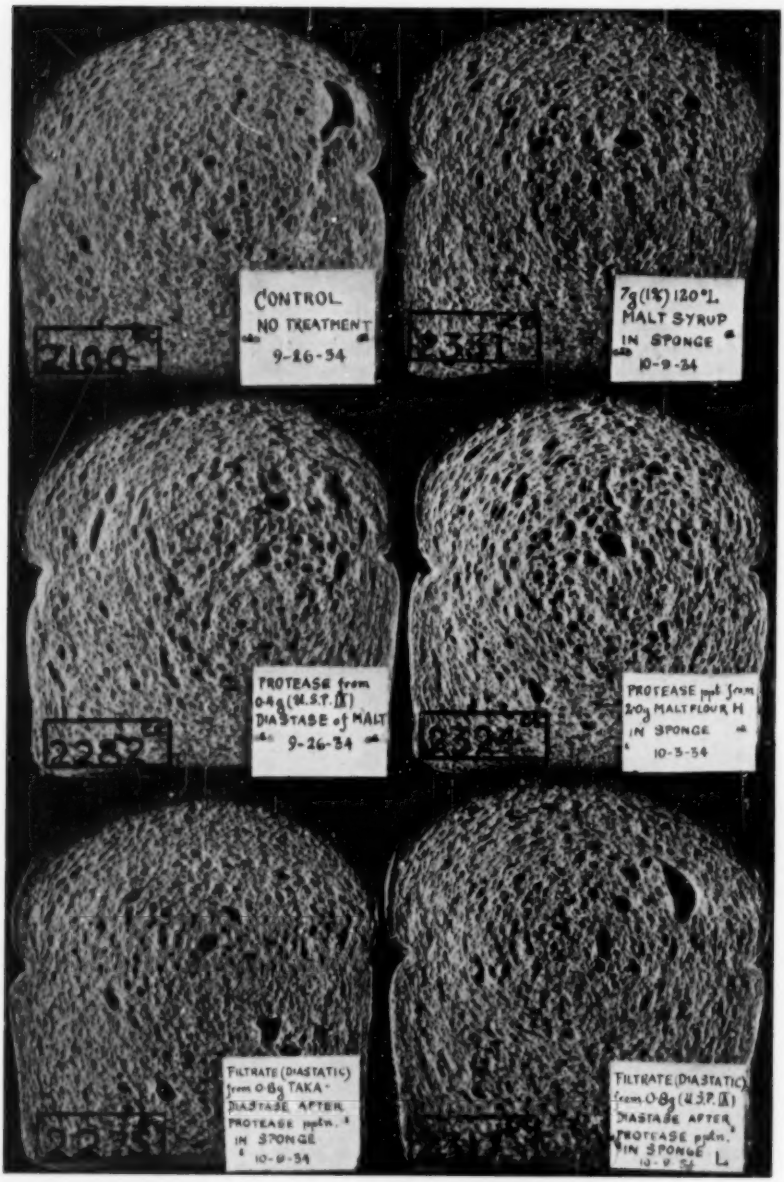

Fig. 6. Baking results with flour No. 129901, showing effects on crumb structure and volume produced by Diamalt (120° L.), small dosages of precipitated protease from diastase of malt and malt flour H, and heavy dosages of filtrate (diastatic) from Taka- and malt-diastase, when added separately to the sponge.

Fig. 7. Baking results with flour No. 126587, showing effects on crumb structure and volume produced by diastase of malt, malt flour H, protease precipitate from these and from Taka-diastase, when added separately to the sponge. It will be noted that the protease precipitated from 10.0 g. of malt flour H was not the equivalent of protease precipitated from 0.6 g. of either Taka-diastase or diastase of malt.

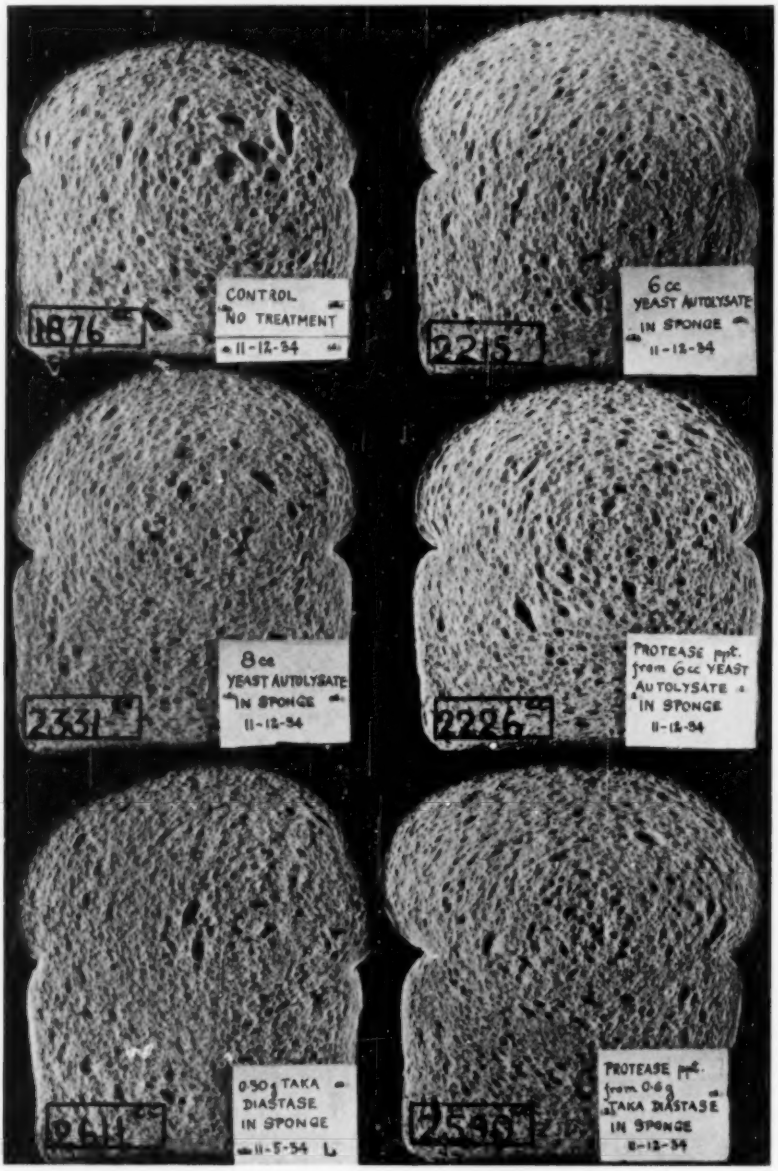

Fig. 8. Basking results with flour No. 126587, showing effects on crumb structure and volume produced by yeast autolysate, Taka-diastase and precipitated protease from these agents when added separately to the sponge.

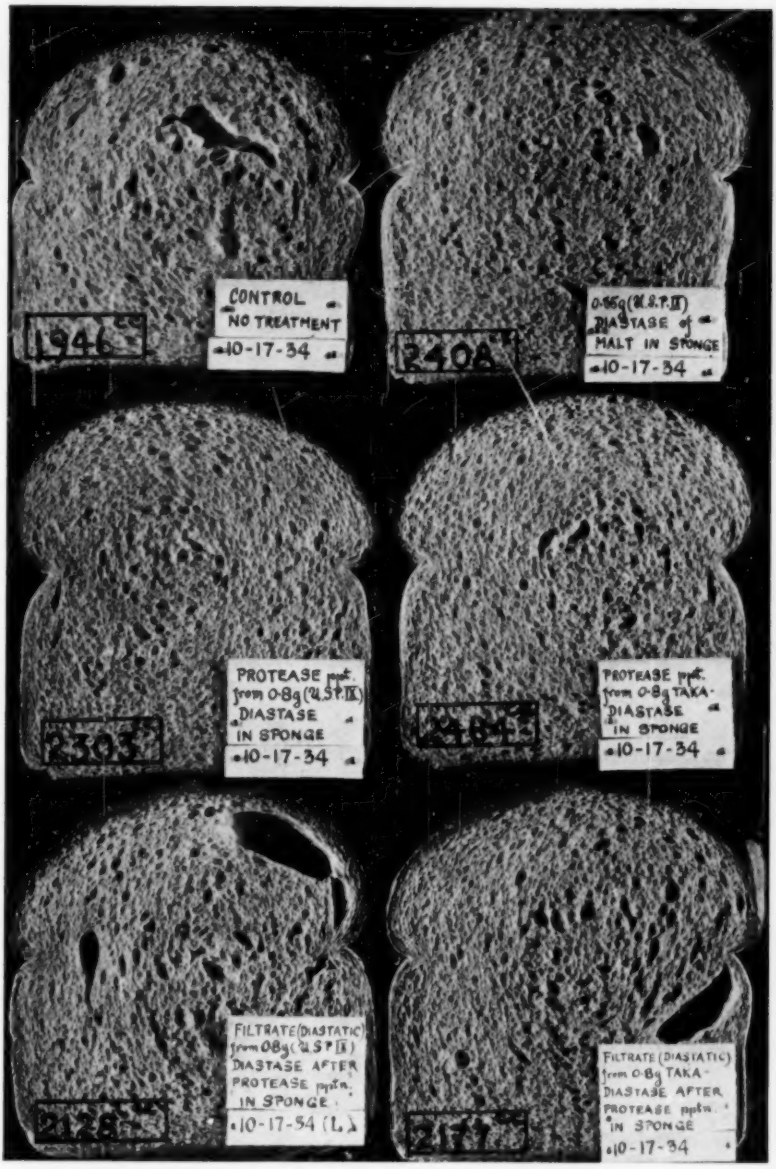

Fig. 9. Baking results with flour No. 128457, showing effects on crumb structure and volume produced by diastase of malt, precipitated protease from Taka- and malt-diastase and corresponding dosages of filtrate when added separately to the sponge.

Summary

Numerous experimental baking tests with different flours giving rise to "bucky" doughs have been presented.

"Bucky" doughs which normally produced bread of low quality were greatly benefited by the addition of small amounts of certain plant proteases in the form of an azo-enzyme precipitate to the sponge. The gluten was sufficiently mellowed to make the dough pliable and lively, thus enabling it to yield loaves of high quality.

The amylolytic activity of the diastatic agents investigated appeared to have little or no value in the matter of dough improvement with the flours investigated. Excessive dosages of amylase produced stickiness.

Commercial products such as Taka-diastase and Merck's diastase of malt (Medicinal-U.S.P. IX) exhibited far more proteolytic activity than did the ordinary diastatic agents which are commonly used in the bake shop.

The relative liquefying power of the different preparations investigated was determined. Taka-diastase failed to respond satisfactorily to the method prescribed by Ohlsson for separating the alpha and beta components of malt amylase.

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SOME METHODS AND APPARATUS USED IN MEASURING THE QUALITY OF EGGS FOR CAKE MAKING

FLORANCE B. KING, H. P. MORRIS, and ELIZABETH FULLER WHITEMAN

Bureau of Home Economics, United States Department of Agriculture, Washington, D. C.

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Introduction

An investigation of egg quality as affected by factors such as diet of the hen, period in laying cycle, and season of the year, is under way cooperatively among the Bureaus of Animal Industry, Chemistry and Soils, and Home Economics. One part of the study is concerned with the effect of these factors upon the lifting power of the eggs when used in food preparation. As a preliminary to a study of this nature there must be studies of measuring not only differences in lifting power, but also variations in physical and chemical constants in the eggs used, which may be correlated with lifting power.

It is known fairly definitely that there are differences in eggs caused by various production factors, but it is not known whether or not these or other differences affect the lifting power of the egg or the quality of cake made with such eggs. The problem has been divided into two parts, first, the measurement of certain physical and chemical properties of the fresh egg, and, second, the quality of cake baked from the same sample of eggs. Attempts have been made to correlate these two types of measurements in an effort to evaluate egg quality in relation to lifting power.

Very little work has been reported in the scientific literature concerning the quantitative measurements of cake quality. Platt and Kratz (1933) made a study of cake as related to egg quality using sponge cake as the test material. Their methods were designed to apply to the commercial type of cake. They have made a marked ad-

vance in the evaluation of cake by devising quantitative measurements other than weight and volume of the finished product, thus replacing the high variability of personal opinion largely used heretofore for measuring cake quality. The methods used in the study here reported are similar in many respects to those used by Platt and Kratz. Certain modifications which have been found desirable are described in sufficient detail to be of use to other workers in this field.

Sources of Eggs

The eggs were obtained from the Bureau of Animal Industry at the Beltsville Research Center of the U. S. Department of Agriculture, Beltsville, Maryland. They were selected as representative of average fresh eggs. Each series of measurements was made from a composite sample including several different groups of hens and, it is believed, therefore, that variabilities due to feed, environment, or other production factors have contributed equally in the different samples studied.

The eggs were collected soon after being laid and were dipped in a liquid mineral oil, saturated with carbon dioxide and having a high pour point. Swenson, Slocum, and James (1932) indicated that oils with high pour points were the most effective for maintaining egg grade. The eggs were delivered to the laboratory within 24 hours after they were laid and then were divided at random. The three dozen day-old eggs received in each sample were used immediately for cakes and one dozen for the physical and chemical measurements.

Physical and Chemical Measurements on the Eggs

Hydrogen-ion concentration. One of the most rapid changes that takes place in the egg during the first few hours after it has been laid is in hydrogen-ion concentration. Sharp and Whitaker (1927) report that the white may change as much as one pH unit during the first 24 hours after laying when held at room temperature. The change in the pH of the yolk is very much less than that of the white. The concensus of opinion at the present time ascribes this rapid change in pH to loss of CO_2, and since, as pointed out above, the greater part of this change in the white occurs during the first 24 hours after the egg was laid, it was believed that dipping the eggs in carbon dioxide saturated mineral oil would greatly retard this change. Swenson and James (1931) have reported that pH of the whites of vacuum oil dipped eggs after 11 months storage averaged 8.20, while open dip averaged 8.63 and unoiled eggs averaged 8.99.

Hydrogen-ion concentration determinations were made with a Bailey hydrogen electrode. The white and yolk were diluted with

redistilled water since the viscosity of the white and yolk is too great for satisfactory use of this electrode. Erickson, Boyden, Martin, and Insko (1932) have shown that dilution does not materially affect the resulting hydrogen-ion determinations.

Table I gives the average values of the hydrogen-ion concentration for white, yolk, and magma. The average pH of 7.95 given for the

TABLE I

CHEMICAL AND PHYSICAL MEASUREMENTS ON EGGS IN THE THREE SERIES

Sample of eggs	pH			Total CO_2 in 100 cc.		Total solids			Viscosity as measured by outflow time
	White	Yolk	Magma	White	Yolk	White	Yolk	Magma	Magma
				Mg.	*Mg.*	%	%	%	*Seconds*
Series 1—Average of determinations on 13 samples of eggs from mature hens	7.90	6.02	7.14	244.5	15.9	11.72	51.96	25.32	34.1
Series 2—Average of determinations on 6 samples of pullet eggs	8.01	6.09	7.24	190.8	10.8	12.95	51.56	24.02	31.6
Series 3—Average of determinations on 11 samples of eggs from mature hens	7.93	6.07	7.09	215.7	13.3	12.43	52.32	27.39 [1]	34.1

[1] Ratio used in cakes (100 grams white to 60 grams yolk).

white indicates that the pH of the egg whites used in these experiments was approximately the same as those reported for eggs less than 3 hours old by Erickson and others. The average pH of 6.05 for the yolk, as given in Table I, was found to agree with the values reported for the yolk of fresh eggs by Baird and Prentice (1930) and by Sharp and Powell (1931), but it is lower than the values obtained by Erickson and others (1932) by about .3 pH unit.

The pH of the magma was intermediate in value between the white and yolk. This would represent the pH of the eggs at the time they were incorporated in the batter for the cakes, since no precautions were taken to prevent carbon dioxide loss. The pH of the magma was always determined within an hour after the eggs had been broken.

Carbon dioxide content. The carbon dioxide content of the whites and the yolks was determined by the Van Slyke manometric blood gas apparatus. One cubic centimeter samples of white and yolk were placed in separate stopcock pipettes immediately after the eggs had been broken and mixed. The CO_2 was determined on these samples within a short time after they had been placed in the pipettes, due precaution being taken to minimize the loss of CO_2 from the time the sample was taken until the CO_2 determination had been carried out. The procedure used was identical to that described for CO_2 in whole blood by Peters and Van Slyke (1932).

The gas absorbing pipette was cleaned after each day's determinations (4 samples) by allowing it to stand overnight filled with approximately 3 to 4% solution of ammonium hydroxide. This dissolved all the egg residues adhering to the walls of the chamber. Before using the pipette was washed once with distilled water followed by one washing with .1 N lactic acid.

Satisfactory determinations could not be made on egg magma owing to the difficulty of obtaining a uniform mixture of white and yolk and to the great differences in CO_2 content of these two portions of the egg and to loss of CO_2 during the mixing. It will be observed from Table I that 92 to 95% of all the CO_2 in the fresh egg is found in the white. Straub and Donck (1934) determining CO_2 in fresh eggs with Geissler's apparatus found no CO_2 in the yolk and an average value of 150.3 mg. CO_2 per 100 cc. of white. Figure 1 shows the rela-

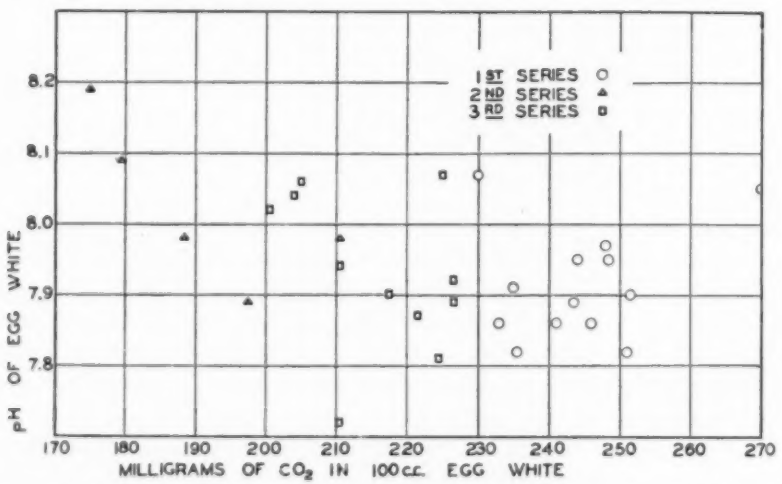

Fig. 1. The relation between the pH values and the CO_2 content of the egg white of the three series.

tionship between the pH values and the CO_2 content of the eggs. Further work must be done on the relation between CO_2 content and

pH before it can be said that the increase in pH of the white is due entirely to a loss of CO_2. Studies are being made on this point.

Total solids. The total solids content of whites and yolks was determined separately in an electric vacuum oven according to the method of the A. O. A. C. (1930), except that the vacuum obtained was never greater than 22 inches of mercury. Comparisons were also made between total solids content determined by this method and by use of the Abbé type of refractometer as described by Almquist, Lorenz, and Burmester (1932). The correlation coefficient between the total solids content by the oven method and by the refractometer was $r = + .995 \pm 0.002$. The linear regression for total solids obtained by this calculation ran parallel to the linear curve given by Almquist and his associates, but yielded a slightly lower value. The refractometer used was checked for accuracy by means of the standard wedge supplied by the manufacturer.

Viscosity. Viscosity of the egg magma was determined according to the method of Thomas and Bailey (1933). A flowmeter was constructed from their specifications. The average values (outflow time) of 31.6 to 34.1 seconds as given in Table I are considerably lower than the values reported by Thomas and Bailey. This difference gives a comparison of the relative viscosity of fresh and frozen egg magma.

Quantitative Measurements of Egg Quality

Cake formula and method of mixing and baking. A true sponge cake was used as a measure of the lifting power of the eggs. A formula was devised that had all the characteristics of a desirable sponge cake. A three-speed Hobart mixer was used with two bowls of 3½ and 10 quarts capacity. The wire egg whip was used for beating the egg whites and the flat beater was used for all other mixing.

The formula used for the sponge cake contained the following ingredients:

440	grams egg white
264	grams egg yolk
308	grams cake flour
540	grams sugar
8	drops lemon oil
5	grams salt
3.4	grams tartaric acid dissolved in 48 cc. distilled water

The sugar was mixed with the egg yolk for 10 minutes and the lemon oil added. The egg white was beaten with the salt and acid solution for 4 minutes at high speed. The yolk-sugar mixture was poured lightly on top of the beaten white-salt-acid mixture and mixed for 10 seconds at low speed. The flour was added in three portions and mixed for 10 seconds after the first two additions and 30 seconds after the last one. All mixtures were kept at 25° C., by means of a

water bath. The formula was of sufficient quantity to make six cakes in pans 7 × 5 inches at the top, 2½ inches deep, and 6½ × 4½ inches at the bottom, with some batter left over, so that it was not necessary to scrape the bowl. Each pan was filled in the order of 1 to 6 with 200 grams of batter in the first two series of bakings. In the third series the pans were filled in three layers in the following order, pan 1, 2, 3, 4, 5, 6; 6, 5, 4, 3, 2, 1; 1, 2, 3, 4, 5, 6. The pans were ungreased and unlined. Platt and Kratz (1933) lined the entire pan with paper, but an earlier study here showed that a concave indentation in the bottom of the cake caused by the paper made an error in the volume measurement.

The 6 cakes were baked in the same relative position each time in a heavily insulated electric oven with thermostatic control, for 14 minutes at 180° C. After removal from the oven the cakes were left in the pans to cool for one hour on a wire rack before being weighed and the volume measured. In Table II are shown the average values of cakes 1 through 6 in the three series of bakings for the following measurements.

TABLE II

THE AVERAGE VALUES OF THE PHYSICAL AND CHEMICAL MEASUREMENTS ON SPONGE CAKE BATTER AND THE FINISHED CAKES—1 THROUGH 6 IN THREE SERIES OF BAKINGS

	Batter		Finished cake			
	Specific gravity	pH	Cake number	Specific volume	Tensile strength	Compressibility [1]
Series I (Average of 13 bakings) Eggs from mature hens	0.362	5.33	1	4.85	24.96	0.65
			2	4.75	26.13	.69
			3	4.71	25.55	.72
			4	4.68	25.33	.69
			5	4.66	25.85	.72
			6	4.54	26.46	.76
Series II (Average of 6 bakings) Pullet eggs	0.373	5.21	1	4.76	25.06	0.73
			2	4.70	24.28	.76
			3	4.64	25.85	.80
			4	4.58	27.35	.84
			5	4.53	27.60	.86
			6	4.53	28.42	.85
Series III (Average of 11 bakings) Eggs from mature hens	0.394	5.19	1	4.64	25.25	0.77
			2	4.69	24.56	.77
			3	4.75	26.20	.79
			4	4.69	25.67	.79
			5	4.68	25.78	.80
			6	4.70	26.60	.79

[1] Expressed in the reciprocal of the measurements actually made. The larger value indicates a more compressible cake.

Specific gravity of the batter. Glabau (1930–1932), Alexander (1931), Platt and Kratz (1933), and others, have determined the specific gravity of cake batter by determining the weight of a standard cupful or of a container of known volume. In this study the weight of batter contained in a crystallizing dish of 185.4 cc. capacity was used to calculate the specific gravity.

Measuring cake volume. Platt and Kratz (1933) and some other investigators have expressed a preference for the planimeter method of measuring the volumes of cakes over the seed displacement method. Platt and Kratz found that the two methods gave comparable results, but stated that "seed displacement methods were not found satisfactory for sponge cake due to the compressibility of the product." The incidental advantages enumerated by Platt and Kratz for the planimeter method are probably of more value to investigations conducted in commercial bakeries than to an investigation such as is described in this paper. It was found that comparative volume measurements made by the seed displacement method are more reproducible than those made by means of the planimeter, and that a series of measurements can be completed more rapidly by this method.

Table III gives the volumes of 6 cakes as determined by the two methods. Columns I to III give the volume of each cake, made in

TABLE III

COMPARISON OF THE DIFFERENCES IN VOLUME MEASUREMENT OF THE SAME CAKES BY SEED DISPLACEMENT AND PLANIMETER METHODS

Cake number	Seed displacement method				Planimeter method					
	I	II	III	Maximum difference	IV	V	VI	VII	VIII	Maximum difference
	Cc.	*Cc.*	*Cc.*	%	*Cc.*	*Cc.*	*Cc.*	*Cc.*	*Cc.*	%
1	908.0	898.7	893.9	1.5	874.4	890.0	899.7	907.6	892.9	3.7
2	884.6	880.8	881.0	.41	857.5	880.3	893.2	915.2	886.7	6.3
3	905.0	901.4	898.5	.72	856.6	869.2	911.9	884.2	880.2	6.1
4	899.3	897.3	896.4	.32	897.9	894.7	886.1	886.1	891.4	1.3
5	897.8	894.9	894.6	.36	895.8	880.9	916.2	914.0	901.4	3.9
6	881.0	876.9	878.8	.47	843.9	851.3	885.4	867.5	862.2	4.7

triplicate for comparative purposes. Columns IV to VIII record the volumes of the same cakes, as determined by the planimeter method. Columns IV and V represent the values obtained by multiplying the cross-sectional areas by the average length of the cake. Outlines were drawn from each of the two halves resulting from each cut. The values reported in columns VI and VII were obtained by multiplying

the longitudinal areas by the average width of the cake. The values in column VIII were obtained by multiplying the area of the cross-section by that of the longitudinal section, and dividing this product by the average thickness. The average thickness was obtained by dividing each of the cross-sectional areas by the average width, and each of the longitudinal sections by the average length. The value in column VIII is thus, in effect, an average of the values reported in columns IV to VII.

In using the seed displacement method in the present study the sides of the baking pans were made high enough so that the measurements could be made in the pan before removal of the cake. After the volume measurement, the cakes, still in the pans, were wrapped in wax paper and stored in tight containers. At the end of 24 hours the volume measurement was repeated. In the first series this measurement was made under ordinary temperatures and atmospheric conditions, in the second series at a temperature of 70° C. and a relative humidity of 65%.

Measurement of tensile strength or toughness. The cake was trimmed to 4″ × 6″ × 1″ in a miter box (Figure 2). Thin metal strips were

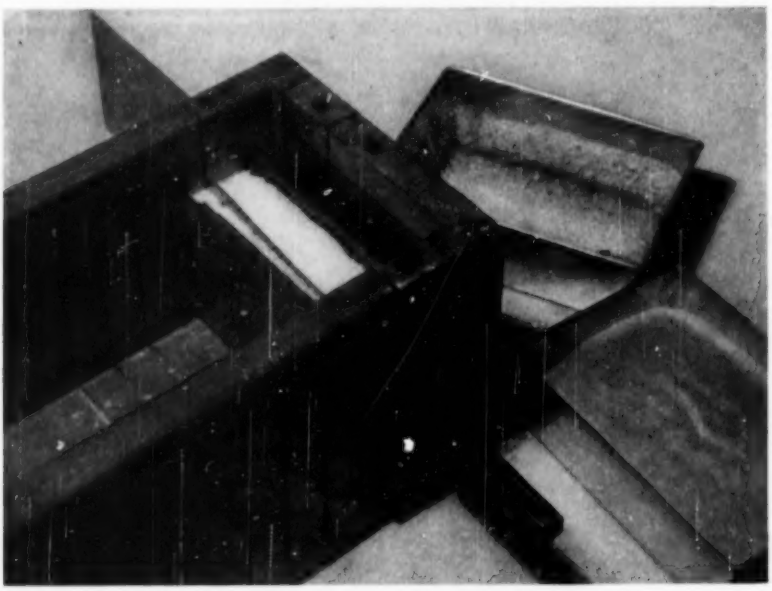

Fig. 2. Miter box used for trimming the cake for measurements.

used to get the exact dimensions. The tensile strength was determined essentially as described by Platt and Kratz (1933) except that a box measuring 4″ × 6″ × 1″ on the inside was used to hold the test

piece of cake while cutting out the indentations (Figure 3). The shape of the top of the box is the desired shape of the test piece. A round cutter 6.4 cm. in diameter was used to make the indentations. The cutter was constructed of monel metal and carefully sharpened. When the indentations were cut it left a neck 3.8 cm. wide.

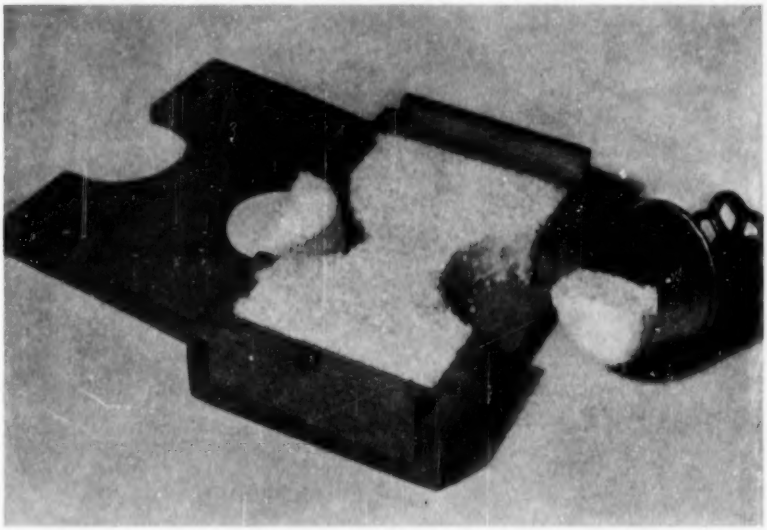

Fig. 3. Box and cutter used to make sample for tensile strength measurement.

Measurement of compressibility or softness. A penetrometer similar to that described by Bonney, Clifford, and Lepper (1931) was used for measuring compressibility of the cake (Figure 4). A cube, 5 cm. on the side, was cut from the center of the piece of cake above the part held by the top spring clamp in the tensile strength measurement. This piece was free from pressure or strain during the first measurement, and its use made possible two measurements on the same cake. The cube was placed under the disk on the floor of the penetrometer. The disk was allowed to come to rest on top of the cake in 30 seconds. The screw which held the disk was then tightened and (*a*) the height of the disk recorded in centimeters from the scale machined on the moveable post which held the disk. The flask was placed on the platform above the screw that was released at the same time the stop watch was started. The stopcock was then turned to allow the mercury to flow into the flask (the combined weight of the flask and mercury was 300 grams). After one minute the screw was tightened and (*b*) the height of the disk recorded. The difference between those two readings (*a*) and (*b*) gave a measure of the compressibility or softness of the test piece.

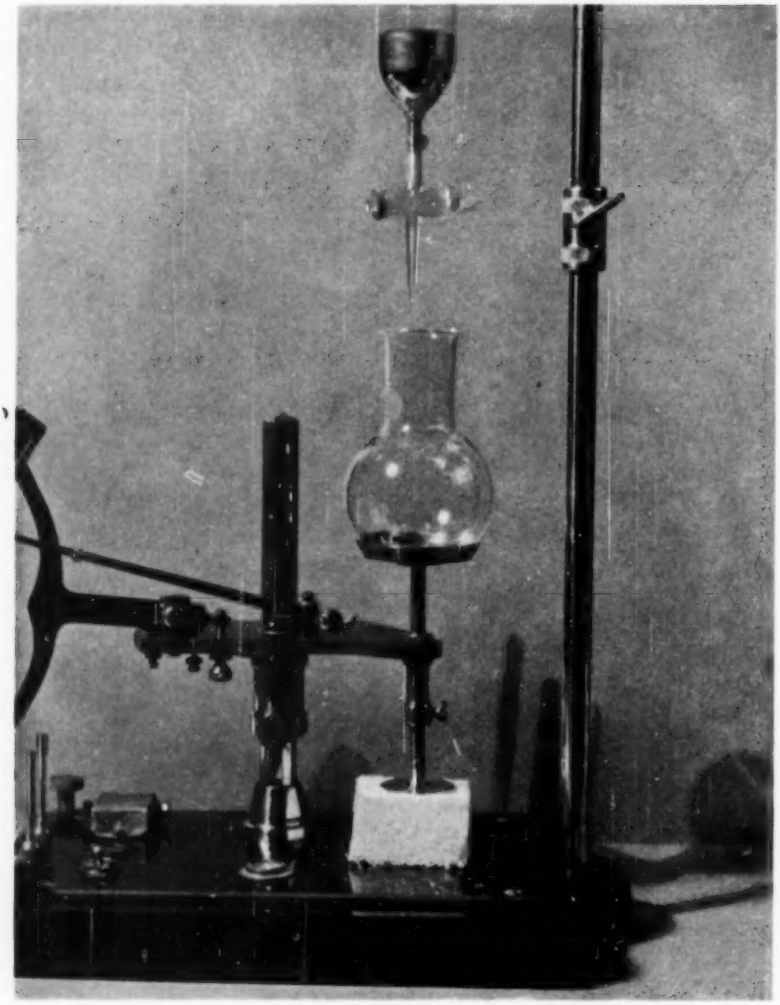

Fig. 4. Apparatus for measuring compressibility of the cake.

Table IV shows the average values of the physical and chemical measurements on 6 cakes in the three series of bakings.

Discussion

In Table II are given the average values obtained in two series of baking tests. The first and second series comprised 19 bakings of 6 cakes each while the third series comprised 10 bakings of 6 cakes each. The batter in the first two series was added to the pan in the order listed while in the third series 1/3 of the amount of batter used was added to each of the 6 pans in rotation. In the first two series the

TABLE IV

AVERAGE VALUES OF THE PHYSICAL AND CHEMICAL MEASUREMENTS ON 6 CAKES IN THE THREE SERIES OF BAKINGS

	Baking number	Batter		Finished cake		
		Specific gravity	pH	Specific volume	Tensile strength	Compressibility [1]
Series I						
Eggs from mature hens	1	0.370	5.30	4.69	27.63	0.68
	2	.378	5.25	4.64	28.26	.68
	3	.352	—	4.83	26.83	.64
	4	.358	5.80	4.85	25.14	.67
	5	.360	5.43	4.59	26.25	.73
	6	.370	5.65	4.55	24.42	.70
	7	.365	4.95	4.66	21.92	.70
	8	.360	4.80	4.60	24.91	.67
	9	.358	5.32	4.70	25.57	.75
	10	.373	5.43	4.50	25.34	.73
	11	.362	5.31	4.76	27.75	.77
	12	.342	5.29	4.85	28.06	.76
	13	.352	5.40	4.80	22.18	.67
Average		.362	5.33	4.69	25.71	.70
Series II						
Pullet eggs	14	.347	5.18	4.89	25.82	.71
	15	.378	5.26	4.69	26.05	.83
	16	.354	5.20	4.64	25.65	.78
	17	.378	5.19	4.54	27.84	.79
	18	.384	5.21	4.54	26.59	.81
	19	.395	5.24	4.44	26.60	.89
Average		.373	5.21	4.62	26.43	.80
Series III						
Eggs from mature hens	20	.384	4.98	4.68	25.59	.76
	21	.368	5.18	4.75	24.92	.76
	22	.406	5.14	4.67	26.80	.77
	23	.381	5.21	4.72	26.82	.80
	24	.394	5.24	4.64	26.63	.81
	25	.407	5.26	4.68	25.11	.78
	26	.380	5.32	4.69	26.12	.81
	27	.402	5.26	4.64	25.83	.81
	28	.411	5.11	4.63	27.76	.81
	29	.412	5.23	4.68	23.20	.76
	30	.384	5.20	4.77	23.66	.75
Average		.394	5.19	4.69	25.68	.78

[1] Expressed in the reciprocal of the measurements actually made. The larger value indicates a more compressible cake.

four quantitative measurements on the finished cake were made in the laboratory without regard to temperature or humidity control; in the third series the measurements were made at a constant temperature and humidity. An inspection of the figures in Table II shows that

there was in the first two series a progressive decrease in specific volume from cake 1 to cake 6, and the cakes became tougher and less soft in the order given. In the cakes of the third series these same measurements are more nearly uniform. All measurements in all the series were made in the same order, *i.e.*, from cake 1 through cake 6.

Both changes in technic as noted above have probably contributed to a greater uniformity of results in the third series of bakings, but owing to the fact that the technic was changed in both respects at the same time it is impossible to evaluate the magnitude of the effect of the modified method of filling the pans or to the use of the constant temperature and humidity room in making quantitative measurements on the finished cakes. Suffice it to say that both precautions should be observed for the most uniform results.

Any set of 6 cakes shows a range in variability even with the most careful manipulation which it is possible to carry out. Therefore, it seems that the only feasible method is to standardize all manipulations, take the measurements in a constant temperature and humidity room, base the results on average determinations of at least 6 cakes, and make a large series of bakings.

The chemical and physical properties of the eggs so far measured have shown no definite relation to the various cake measurements.

Summary

The detailed methods for measuring the lifting power of eggs are based on quantitative measurements of specific volume, tensile strength, and compressibility of sponge cakes.

Most uniform results were obtained when the cake batter was added to the pans 1/3 at a time in rotation, followed by making the quantitative measurements on the finished cakes in a room kept at constant temperature and humidity. The measurements of hydrogen-ion concentration, total carbon dioxide of white and yolk, viscosity of magma, total solids of white and yolk of fresh eggs do not appear to be definitely related to the quantitative cake measurements.

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THE MECHANISM OF DOUGH FERMENTATION: NOTE ON A METHOD FOR COUNTING YEAST CELLS IN A FERMENTING DOUGH

A. G. SIMPSON

Associated London Flour Mills Limited, Deptford Bridge Mills, London, England

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The question as to whether any growth of yeast may occur in a fermenting dough, is one that has received little attention, perhaps because no suitable methods have been available for the counting of yeast cells in dough.

Turley [1] has discussed various methods for this determination, and has described a method in which the dough is digested with pepsin-hydrochloric acid to disintegrate the gluten; the yeast cells, after staining with methylene blue, being counted in the suspension obtained, using a haemacytometer.

In practice, particularly when working with doughs containing small percentages of yeast, the actual counting of the yeast cells was found to be a difficult and tiresome operation, as the field was apt to be obscured by starch granules.

To overcome this difficulty, the digested dough suspension was neutralised with alkali, boiled to gelatinize starch, cooled and digested with the addition of diastatic malt extract; the yeast cells were stained with methylene blue and counted in the haemacytometer, as in Turley's method.

It was found that this procedure did not destroy the yeast cells, and the removal of the starch granules enabled the yeast cells to be easily counted.

Method

The method finally adopted was as follows:

The dough from, say, 20 g. of flour, was rapidly cut up into small pieces, put into a 600 cc. conical flask with 400 cc. of 0.05 N HCl containing 0.2% of pepsin scales, and placed in a water bath maintained at 40° C. The flask was repeatedly well shaken during digestion which generally took 45 minutes to 1 hour, and the shaking was continued until all small pieces of dough had been dispersed. A few drops of bromthymol blue indicator were added, and the liquid titrated with normal Na_2CO_3 to a greenish-blue colour. The flask was placed in a boiling water bath, being shaken at first till the temperature of its contents rose to 90° C., after which it remained in the bath for an additional five minutes. After cooling the liquid to 65° C., 10 cc. of a 5% dilution of a diastatic malt extract (about 100° Lintner) were added, digestion being continued for 10 minutes at 65° C. After

[1] Turley, H. E. Counting yeast cells in dough. Cereal Chem. 1: 261–267 (1924)

cooling to room temperature 1 cc. of 1% methylene blue solution was added, the flask well shaken, and its contents transferred to a 500 cc. graduated flask, the washing from the conical flask being used to make up the volume to 500 cc.

After appropriate dilution of an aliquot portion, if necessary, the yeast cells were counted in a Leitz haemacytometer in the usual way.

The following experiments show that with this method no destruction of yeast cells occurs.

Experiment 1

(*a*) 10 cc. of a 1% yeast suspension were diluted to 100 cc. with water, and the yeast cells counted in the haemacytometer.

(*b*) A further 10% dilution of the same 1% suspension was made, and the yeast cells counted.

(*c*) 10 cc. of the same suspension were diluted to 100 cc. with 0.2% pepsin in N/10 HCl, the suspension being then maintained at 40° C. for 1 hour. The yeast cells were then counted.

(*d*) Duplicate of (*c*) on the same original 1% suspension.

144 small squares (each equivalent to 1/4000 cu. mm.) were counted, giving the following average number of cells per standard volume (1/4000 cu. mm.):

	Not digested		*Digested*
(*a*)	4.6	(*c*)	4.8
(*b*)	5.05	(*d*)	4.6
Average	4.8	Average	4.7

As found by Turley, peptic digestion causes no destruction of yeast cells.

Experiment 2

The experiments were repeated by diluting 4 cc. of a 1% yeast suspension to 100 cc. (giving 0.04% of yeast in the final dilution), and, secondly, digesting 4 cc. of the same 1% yeast suspension with 50 cc. of pepsin-hydrochloric acid for 1 hour at 40° C., neutralising with normal Na_2CO_3, placing in the boiling water bath for 10 minutes, cooling to 65° C., adding 5 cc. of a 10% dilution of malt extract, maintaining at 65° C. for 10 minutes, cooling and diluting to 100 cc. Three series of counts were made as follows:

(*a*) 5 slides using the undigested 0.04% suspension were counted.

(*b*) 5 slides using the digested 0.04% suspension were counted.

(*c*) 4 slides using a second digested 0.04% suspension were counted.

144 small squares gave the following total numbers of cells:

(*a*) 175, 153, 215, 139, 165	average of 2.1 cells per standard volume
(*b*) 156, 183, 152, 153, 153	average of 2.0 cells per standard volume
(*c*) 154, 166, 168, 151	average of 2.0 cells per standard volume

Again, the combined peptic and diastatic digestion caused no appreciable destruction of yeast cells.

Experiment 3

In a third experiment, 10 cc. of a 1% yeast suspension was taken, diluting finally to 100 cc. 1 gram of wheat starch was added at the beginning in the case of the digested suspension, the procedure as regards the rest being the same as described in Experiment 2.

The counting procedure was as follows:

(*a*) Undigested suspension, 3 slides counted.
(*b*) Digested suspension, 4 slides counted.

144 small squares gave the following counts:

(*a*)	603, 720, 584	average of 4.4 cells per standard volume
(*b*)	711, 620, 711, 666	average of 4.7 cells per standard volume

Again the combined peptic and diastatic digestion in the presence of starch, caused no destruction of yeast cells.

Experiment 4

In a fourth experiment, carried out in the same manner as Experiment 3, with the exception that 1 gram of wheat-flour was substituted for wheat-starch, and the final dilution of yeast obtained was 0.014%. The following counts were obtained:

(*a*) 5 slides were counted in the case of the undigested suspension, giving an average count of 0.49 cell per standard volume.
(*b*) 10 slides in the case of the digested suspension gave 0.49 cell per standard volume.

Experiment 5

In a repetition of Experiment 4 with the exception that a 0.02% suspension of yeast was finally obtained, two separate dilutions of the undigested suspension were made up and 5 slides were counted from each dilution (*a*), while in the case of the digested suspension, four separate preparations were made, 6 slides being counted from each preparation (*b*).

(*a*)	123, 115, 104, 112, 145	average of 0.8 cell per standard volume
	94, 119, 124, 100, 127	average of 0.8 cell per standard volume
	General average of 0.8 cell per standard volume	
(*b*)	94, 102, 119, 101, 101, 116	average of 0.74 cell per standard volume
	114, 121, 126, 121, 112, 98	average of 0.82 cell per standard volume
	113, 125, 120, 88, 114, 85	average of 0.75 cell per standard volume
	115, 97, 87, 121, 118, 88	average of 0.72 cell per standard volume
	General average of 0.76 cell per standard volume	

Experiment 6

20 grams of wheat flour were made into a dough with 10 cc. of a 2% yeast suspension, rapidly cut into small pieces, and placed in a flask with 400 cc. of 0.2% pepsin in 0.05 N HCl. After digestion for 1 hour the volume was made up to 500 cc. 50 cc. of the well shaken suspension were neutralised as described above, boiled and digested with 10 cc. of a 5% malt extract, the volume being finally made up to 100 cc. This final suspension therefore contained 0.02% yeast (6*b*). Ten slides were counted.

For the undigested suspension 10 cc. of the original 2% yeast suspension were diluted to 1 litre with water, giving a 0.02% suspension. Five dilutions from the original 2% suspension were made, 5 slides from each dilution being counted (6*a*).

(*a*)	105, 90, 102, 125, 112,	average of 109, or 0.74 cell per standard volume
	97, 112, 85, 115, 104,	average of 103, or 0.71 cell per standard volume
	78, 89, 85, 105, 74,	average of 86, or 0.60 cell per standard volume
	101, 112, 83, 101, 129,	average of 106, or 0.74 cell per standard volume
	105, 99, 88, 93, 96,	average of 97, or 0.67 cell per standard volume
	General average of 100, or 0.69 cell per standard volume	
(*b*)	77, 90, 86, 92, 107	average of 100, or 0.69 cell per standard volume
	106, 113, 110, 105, 114	average of 100, or 0.69 cell per standard volume

Experiment 7

Doughs were made up from 20 grams of two different flours, with 11 cc. of a 2% yeast suspension, cut up into small pieces as quickly as possible, the rest of the procedure being as already described. The final suspension was made up to 1000 cc.

11 cc. of the same 2% yeast suspension were made up to 1000 cc., as the control.

The three doughs were made up on three different days; in each case three slides were counted, from the control and from the digested dough suspension.

	Yeast cells per standard volume [1]	
	Dough	*Control*
Flour No. 1	1.00	1.16
Flour No. 2	0.97	1.05
Flour No. 3	1.30	1.30

[1] The number of yeast cells in one gram of yeast, calculated from the data given above, is on the average 1.72×10^{10}, ranging from 1.38×10^{10} to 2.02×10^{10}.
From the figures given in Turley's paper, the calculated number of cells per gram of yeast would be 7.87×10^{9}.

Summary

A method for counting yeast cells in dough, based on the method of H. E. Turley, is described.

The dough is dispersed by digestion with pepsin-hydrochloric acid, as in the Turley method, neutralised with alkali, boiled and digested with diluted diastatic malt extract, and the yeast cells stained with methylene blue.

The yeast cells are easily counted in the haemacytometer, a perfectly clear field free from starch granules being obtained.

No destruction of yeast cells results from this procedure.

PROTEOLYTIC ENZYMES OF FLOUR

A. K. Balls and W. S. Hale

Bureau of Chemistry and Soils, U. S. Department of Agriculture, Washington, D. C.

(Paper presented before the American Association of Cereal Chemists, June 8, 1935, at Denver, Colorado)

In spite of considerable attention from biochemists, the proteolytic enzymes of flour have remained uncharacterized and little known. This is not due to a lack of appreciation of their possible significance, but rather to the experimental difficulties incident to their study. According to Cairns and Bailey (1928) proteinases are present in flour only in very small amounts and have rarely been separated from the other constituents.

Recently Balls and Hale (1935) described a new method for estimating the proteolytic enzymes in flour. The method amounts essentially to the autolysis of the flour proteins by the proteinase that accompanies them, and measurement of the quantity of water-soluble amino nitrogen, as increased during the digestion, by titration in alcohol. The method is by no means perfect, but it appears to us that by using fat-extracted flour, it avoids one difficulty not heretofore taken into account in titration methods, namely, the production of acid by the action of the flour lipase.

The possible error from this source might on occasion be serious, for 5 g. of a flour containing 1% of fat could yield ultimately, in the presence of a lipase, fatty acids equivalent to something like 2.0 cc. of 0.1 N KOH. This is a higher reading than the proteinase itself usually produces. We found that after 20 hours' digestion at 40° in the presence of thymol, 2 g. of a flour from low weight wheat in 10 cc. water gave an increased titration for water-soluble amino nitrogen by the alcohol method amounting to 2.50 cc. of 0.1 N KOH; the same flour after removal of the fat with petroleum ether gave an increased titration of only 1.0 cc. of 0.1 N KOH.

These measurements are on the water-soluble fraction only, but results of similar trend were obtained on suspensions of the flour as well. In the case of suspensions it is necessary to use some form of the formol titration (see Table III). Two grams of the same flour cited above when suspended in 10 cc. water gave an increased formol titration of 2.20 cc. 0.1 N KOH in 20 hours at 40°; after extraction of the dry flour with petroleum ether it gave an increase of only 1.70 cc. 0.1 N KOH. Evidently something besides proteolysis occurs in the fat-containing flour to increase the values obtained by these titration methods.

The results so obtained amply confirm the conclusions previously reported by Stockham (1920) and others that the proteinase, like other enzymes in grain, is definitely concentrated about the germ and outer layers of tissue. Therefore, white flour usually contains very little of the enzyme, whole wheat flour contains much more, while both bran and germ contain comparatively large quantities. This is evident from the data shown in Table I.

TABLE I

AUTOLYSIS OF THE PROTEIN IN VARIOUS TISSUES OF WHEAT. INCREASED VALUES ON TITRATION IN ALCOHOL AFTER 2 HOURS AT 40° C. ON A PORTION EQUIVALENT TO 2 GRAMS OF MATERIAL
(see Balls and Hale, 1935)

Wheat tissue	N/10 KOH
	c.c.
White flour	0.45
Whole wheat flour	1.60
Bran	1.70
Germ	2.50

It is not safe to assume that the observed splitting of the flour proteins is so small as to be of no consequence in bread-making. Very considerable modification of a protein can occur and still be recorded as only a small numerical increase in the values measured as the acidity titratable in alcohol, or as amino nitrogen. It is only when the proteins are broken down to peptones and peptides—namely, by a very extensive digestion—that the measurements become numerically large.

In bread-making, however, yeast is also present, and in view of the large amount of proteinase in yeast as compared with flour consideration was given to the possibility that the yeast proteinase completely overshadows the flour enzyme and makes the effect of the latter meaningless from a practical standpoint. This is not the case, however, for the reason that the yeast proteinase remains confined within the yeast cells. The work of Willstätter and Grassmann (1926) indicates that unless the yeast cells break up, the proteinase is not liberated. That it can have no marked effect upon the flour proteins is evident from the work of Cairns and Bailey (1928) and also follows from the fact that ordinary yeast cultures liquefy gelatine very slowly, when at all.

The proteinase of flour was shown by Cairns and Bailey (*loc. cit.*) to be soluble in water. For the purpose of examining the enzyme in solution and thus apart from the main bulk of flour constituents, an extract with dilute alkali was found by us to be more satisfactory. This extract was conveniently made from bran.

After soaking the bran in sufficient cold and very dilute ammonia to maintain a pH of 9 in the mixture, the liquid was squeezed out of the mass, neutralized, and centrifuged to free it from most of the suspended matter. Some of the proteinase remains in this solution. Such preparations are neither pure nor stable, but they suffice to determine some important properties of the enzyme. The proteinase is usually inactivated by standing over night in the air, probably through a process of oxidation. On the other hand the enzyme is activated by cysteine hydrochloride and glutathione. The activating effect of cysteine and similar substances on the dissolved enzyme confirms our previously reported conclusion (Balls and Hale, 1935) that this proteinase of wheat belongs

TABLE II

ACTIVATION OF WHEAT PROTEINASE BY CYSTEINE OR GLUTATHIONE

A. Autolysis of solid material for 2 hours at 40° C.

Material	Increase in titration values [1]	
	No additions	With 1.5 mg. cysteine per gram of material
	Cc. N/10 KOH	*Cc. N/10 KOH*
Wheat germ	2.50	3.00
Whole wheat flour (*a*)	1.95	2.40 (with glutathione)
Whole wheat flour (*b*)	0.70	1.15
Wheat malt	3.00	3.15
White flour	0.45	0.75

B. Enzyme extracted from bran.[2]

Enzyme preparation	Time	Increase in titration values [1]		Substrate
		No additions	With cysteine or glutathione	
	Hours	*Cc. N/10 KOH*	*Cc. N/10 KOH*	
A	20	0	0.25	Old inactive white flour
	2	0.15	1.70	Casein
B	2	0	0	No substrate
	20	0.45	1.95	Casein
	20	0	0	No substrate
C	20	0.85	0.95	Casein (glutathione)

[1] See Balls and Hale, 1935.

[2] 5 cc. enzyme, equivalent to 1.0 g. bran; substrate 0.3 g.; temp. 30° C.; time as shown; pH = 6; cysteine·HCl 25 mg.

to the class of proteolytic enzymes known as papainases. The activation is shown in Table II.

Besides furnishing a means of identifying the proteinase of flour, the activating effect of sulphydryl compounds has practical significance. That a reducing system similar in its action to glutathione or cysteine is already present in flours follows from the fact that the untreated flour shows some enzymic activity. When added to bread dough, an additional quantity of either enzyme or activator produces a striking result, as shown in Figure 1. In either case the dough is practically liquefied, and the loaf does not rise at all.

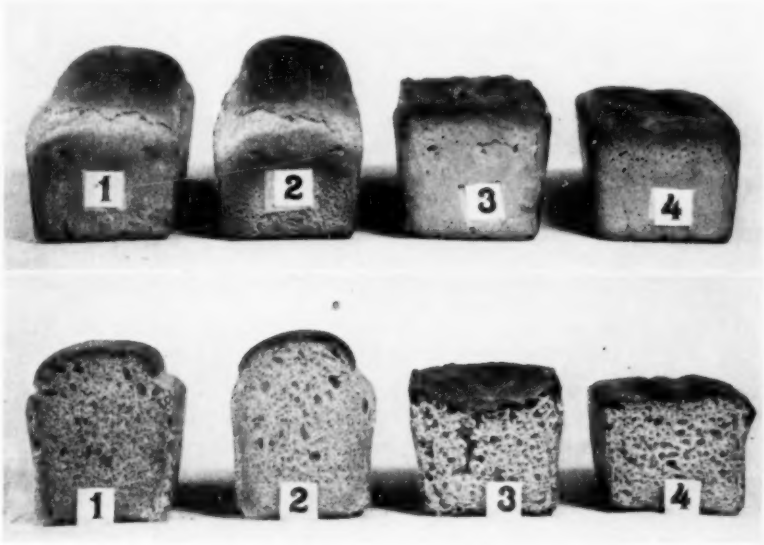

Fig. 1. One pound loaves of bread containing the additions shown.[1]

Oxidizing agents, on the other hand, oxidize the naturally occurring activator of the flour proteinase and therefore reduce the proteolytic activity in the dough. Such doughs ought to be tougher and less fluid. It is common knowledge that oxidation by bleaching, by the use of bread improvers such as persulphate, and even by simple storage in the air produces this change in the properties of the flour. Kent-Jones (1927) by the use of viscosity measurements concluded that the aging of flour markedly reduces its proteolytic activity. The chemical mechanism by which oxidation produces this result, however, is first apparent from the

[1] We are greatly indebted to J. A. LeClerc and L. H. Bailey of the Cereal Laboratory of this Division for undertaking the baking experiments for us. The picture shows a series of loaves baked on May 27, 1935. Mr. Bailey has informed us that small quantities of fresh pineapple juice gave results similar to those shown here with papain.

TABLE III

DECREASE IN THE PROTEOLYTIC ACTIVITY OF FLOUR CAUSED BY BLEACHING OR STORAGE IN AIR [1]

Treatment	Material	Increase in 20 hours in—	
		Amino N.	Formol titration on suspension of flour
		(*mg. N. per g. flour*)	(*Cc. N/10 KOH per g. of flour*)
	Whole wheat flour (about 60 days old) [2]		
1	Untreated	0.17	1.83
2	Untreated + 10 mg. glutathione per g. of flour	0.60	
3	Bleached with 1 g. Cl_2 per kg. of flour	0.11	
4	Bleached with 1 g. Cl_2 per kg., then + 10 mg. glutathione per g.	0.55	
5	Bleached with 2 g. Cl_2 per kg. of flour	0.06	1.22
	White flour		
6	Fresh	0.50	
7	After storage, 3 weeks at room temp.	0.33	
	White flour, 5 months old		
8	Untreated	0.27	0.63
9	Bleached with 1 g. Cl_2 per kg. of flour	.0	0.52
10	Bleached with 2 g. Cl_2 per kg. of flour	.0	
11	Bleached with 2 g. Cl_2 per kg, then + 50 mg. glutathione per g.	.0	
	White flour from 40 lb. wheat		
12	Untreated	0.34	1.70
13	Bleached with 1 g. Cl_2 per kg. of flour	0.39	0.80
14	Bleached with 2 g. Cl_2 per kg. of flour		0.65
	White flour from 60 lb. wheat		
15	Untreated	0.22	0.85
16	Bleached with 1 g. Cl_2 per kg. of flour	0.23	0.70
17	Bleached with 2 g. Cl_2 per kg. of flour		0.85

[1] Amino nitrogen determined in the volumetric Van Slyke apparatus was measured on flour saturated with CO_2 while dry, then suspended in water in the proportion of 1 gram in 5 cc. The increase in amino nitrogen after 20 hours' digestion at 30° C. is reported from analyses made on 5-cc. portions of the suspension (= 1 gram whole flour), thymol being present.

Formol titrations were made on flour previously freed from fat by petroleum ether. Twenty grams of flour was suspended in water containing 3 cc. 0.1 N HCl in a total volume of 100 cc., thymol being used as preservative. The increase after 20 hours at 40° C. is reported from titrations made on 10 cc. of flour suspension titrated with N/10 alcoholic KOH in 250 cc. 95% alcohol 15 minutes after the addition of 25 cc. of 40% formaldehyde.

[2] Sieved through a 40 mesh screen.

fact that the proteinase in question requires a reducing substance to activate it. Table III shows that as a result of bleaching with chlorine or of continued storage, the active proteolytic power of the flour is decreased.[1] That the action is a true proteolysis is also evident from the

[1] So little proteinase is present that unfortunately the differences determined by the Van Slyke machine are sometimes but little greater than the probable accuracy of the apparatus in use on such an unsatisfactory material as a suspension of flour. The results would therefore be open to question if they were not corroborated by the formol titration. The latter method may possibly measure amino groups not directly on the surface of the flour particles, and not immediately accessible to the nitrous acid solution. The determination of water-soluble amino nitrogen does not differentiate as well as the formol titration between bleached and unbleached flour, because sometimes the chlorine treatment alone appears to increase the solubility of the nitrogen-containing substances.

increased values for amino nitrogen obtained after the addition of traces of cysteine, or glutathione. The potential proteolytic power, *i.e.*, after reactivation with glutathione, is not damaged correspondingly, unless the flour has been very vigorously oxidized (see Table III, treatments 2, 4, and 11). Usually the reaction is, to a great extent, reversible. High grade white flour, which contains very little proteinase to start with, shows correspondingly less decrease on bleaching (see Table III, treatments 15, 16, and 17).

A Supplementary Method for Determining Proteinase

Another method of differentiating between the proteinase content of bleached and unbleached flour consisted in adding a small quantity of the dry flour to a large quantity of casein solution at a pH of 5.8, and thereafter measuring the breakdown of the proteins by the usual alcoholic titration method for casein digestion (Balls, Swenson, and Stuart, 1935). The amount of flour present was too small to interfere with the

TABLE IV

DIGESTION OF CASEIN BY FLOUR [1, 2]

Treatment	Increase in cc. 0.1 N KOH observed after 48 hours digestion at 40° C.
	Cc.
Flour suspension + buffer—no casein	0.00
Casein solution + buffer—no flour	0.00
Casein solution + buffer—no flour— + glutathione	0.00
Casein solution + untreated flour	0.10
Casein solution + untreated flour + glutathione	0.25
Casein solution + bleached flour	0.00
Casein solution + bleached flour + glutathione	0.15

[1] Hammarsten casein was dissolved in dilute cold NaOH, and enough M/5 citrate — HCl buffer (pH 2.2) was added to bring the casein solution to pH 5.8, where it is about ready to precipitate. The concentration of casein in this solution was about 6%. 240 mg. of flour was added to 15 cc. of casein solution, then sufficient water was added to make 20 cc. Portions of 5 cc. were removed for titration at the start and after 48 hours. Titrations were made in alcohol by the method described in detail by Balls, Swenson, and Stuart (1935). The values shown in the table therefore refer to the effect of 60 mg. of flour on 220-mg. casein. Glutathione when used was added directly to the mixture in a total amount of 10 mg., therefore 2.5 mg. per portion used for titration.

[2] See Table III, items 8 to 11 for flour characteristics.

usual conduct of the titration. The results, shown in Table IV, corroborate those recorded in Table III. Some return of activity was observed in the flour totally inactivated by a heavy dose of chlorine, but it was not apparent until after more than 24 hours digestion. The long digestion time (2 days) required by this method is unfortunate, and is not to be recommended, even though apparently adequate preservative effects were obtained from the added thymol.

Conclusions

During the process of dough fermentation the action of a proteinase in flour changes the (colloidal) properties of the wheat proteins. The change in the flour proteins will eventually show itself in the properties of the "gluten." Proteinases usually produce first a coagulation of the protein; later the coagulated material is broken down and perhaps ultimately dissolved. If this rule holds for flour, in the first phase of proteinase action the gluten would probably become more tenacious; in the second phase it would be broken down to a thinner, more nearly liquid material. It is to be expected, therefore, that a small amount of proteinase in flour would be beneficial. Aside from this theory, a small amount of proteinase is beneficial in any case, for the reason that a flour containing no proteinase at all would form a dough altogether too stiff and inelastic. A large amount of proteinase would, on the contrary, be most harmful. It is evident that a very small amount of the proteolytic enzyme is ample to produce the desirable effects. Usually there is too much, rather than too little.

It is now possible to give a satisfactory explanation of the changes that take place in the quality and behaviour of flours after they have been bleached or stored in air. This alteration of flour is due to a diminution of the proteolytic activity, brought about by the oxidation of the activator of the flour proteinase. Similar effects are produced when the oxidant is added to the dough as a "bread improver."

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MINERALS OF WHEAT—II. THE DETERMINATION OF SODIUM AND POTASSIUM

M. Allison Howe and B. Sullivan

Russell-Miller Milling Company, Minneapolis, Minnesota

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In a preceding paper from this laboratory (Sullivan and Near, 1927), it was stated that some of the minerals of wheat (phosphorus, calcium, magnesium, iron, manganese, etc.) are not lost during the ordinary ashing process, and from an ash analysis an accurate calculation can be made of the total amounts of these elements in the product. On the other hand, it has been shown that certain elements such as sulfur and chlorine are almost completely lost upon ignition of samples at 590° to 600° C., and in order to obtain a correct estimation of the minerals of wheat, it is necessary to measure such elements in the product itself by procedures which will prevent any losses by volatilization (Sullivan and Howe, 1929). In this article sodium and potassium will be discussed. The amount of sodium in the ash of wheat and wheat products has been reported in the literature as varying from none (Teller, 1896) to as high as 9.55% sodium oxide (Bull. 13, U. S. D. A., 1898). It is assumed that in all cases both sodium and potassium were determined by the use of the well known chloroplatinate method, the sodium being determined by difference from the weight of the mixed chlorides.

Experimental

Materials: A hard spring Marquis wheat of high protein content and the separations made from it were taken for analysis. Ash was determined by heating in an electric muffle at 590° C. for 16 hours.

Method: The development of accurate methods for the determination of sodium and potassium both in the product and in the ash presented a considerable problem. In the case of wheat, as with many other cereals, potassium is present in large excess over sodium which occurs in very small amounts. The measurement of these small amounts of sodium by difference from the weight of the mixed chlorides by either the standard-perchloric-acid method or the chloroplatinate procedure, as is commonly done, was not found to be satisfactory, as erratic results were obtained. Potassium determinations gave more satisfactory re-

sults as the potassium was present in larger amounts and was determined directly. A number of methods for both sodium and potassium were tested. The following direct methods are given in detail as they were found to give good results and furnish much more reliable information on the sodium and potassium content of wheat products than any of the previous standard methods which were tried by the authors. So far as is known, no studies have been made on the possible loss of sodium and potassium during ashing. It has been generally assumed by many workers that no loss of either of these constituents takes place during the ashing procedure commonly employed in routine work.

Determination of Potassium in Ash

Potassium was determined on the ash obtained from 5 to 50 g. of each product weighed accurately in a platinum dish, the weight being varied in order to give about 0.2 g. ash. The resulting ash was then heated with 25 cc. of water and 3 cc. concentrated HCl, filtered, washed with hot water, and the sulfates precipitated with barium chloride. The filtrate was evaporated to 25 cc., transferred to a platinum dish, 2 cc. of 60% perchloric acid were added, and the contents evaporated to a sirupy consistency on a water bath. The sample was then transferred to a sand bath and heated until the appearance of white fumes. 15 cc. of hot water and 1 cc. of perchloric acid were added and the liquid again evaporated until the appearance of white fumes. Again 15 cc. of hot water were added and the sample evaporated in the same manner. All of the HCl can be removed by repeated evaporations with perchloric acid. Phosphates need not be precipitated, but sufficient perchloric acid should be used to insure the removal of the phosphates as H_3PO_4 by the wash liquid. The mixture was allowed to cool and then taken up in 20 cc. of 97% alcohol containing 0.2% by weight of 60% perchloric acid (1.7 cc. of 60% acid in 1 liter of alcohol) and decanted through a weighed platinum Gooch crucible. The remaining alcohol was driven off on a water bath and 15 cc. of hot water containing a few drops of perchloric acid added and the mixture again evaporated until the appearance of heavy fumes. The mixture was taken up in a few cubic centimeters of wash liquid and filtered through the Gooch crucible, the precipitate washed with acid alcohol and finally with 2 cc. of pure 97% alcohol. The crucible was dried at 130° C. for one hour and weighed.

Determination of Potassium in Wheat Products

Potassium was determined on the product by a wet digestion method. From 5 to 25 g. of product were refluxed for 5 hours with 25 cc. of

perchloric acid and 50 cc. of concentrated nitric acid in a Vitreosil flask (Figure 1). The liquid was then transferred to a beaker and evaporated to dryness on a sand bath, taken up in hot water and the sulfates precipitated. The filtrate was evaporated to 25 cc., transferred to a platinum dish, and the same procedure for the determination of potassium, as outlined above, was followed. Blanks were determined on all reagents used.

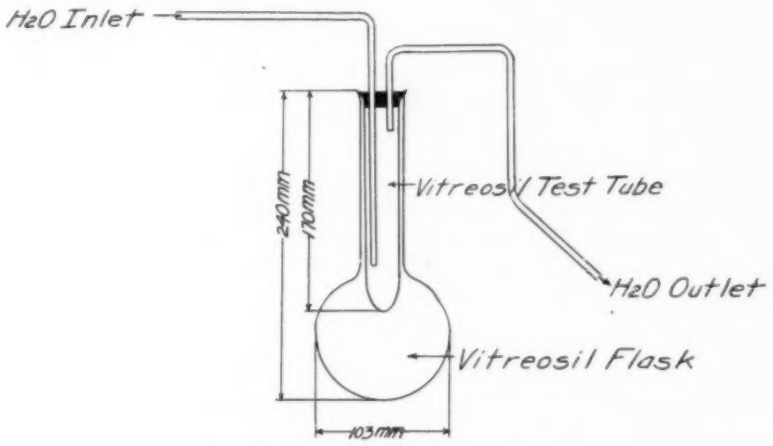

Fig. 1. Apparatus for use in determination of potassium in wheat products.

Determination of Sodium

The identification of sodium by the use of magnesium uranyl acetate was suggested by Streng and was later studied as a quantitative method by Blanchetière (1923), Bertrand (1929), Caley and Foulk (1929), and others. The method suggested by Bertrand was not found to be highly satisfactory. In his directions Bertrand recommends the removal of phosphates by the use of solution " A " (uranium acetate) and then centrifuging and filtering. This was found to give low results as part of the sodium which was adsorbed on the surface of the gelatinous precipitate was very difficult to remove quantitatively by washing. The removal of phosphorus by precipitating with magnesia mixture also proved unsatisfactory. Phosphorus was removed most effectively by the use of powdered calcium oxide.

Among the direct quantitative determinations of sodium recently reported in the literature, the volumetric method of Dobbins and Byrd (1931) was found to be suitable for the small amounts of sodium present in wheat products. The reagents were made up as follows:

Solution A	Uranyl Acetate, c.p.	85 g.
	Acetic acid 95%	50 cc.
	Water	400 cc.
Solution B	Zinc Acetate, c.p.	200 g.
	Acetic acid 95%	25 cc.
	Water	250 cc.

The solutions were heated, mixed and kept at the same temperature as that at which the sodium was precipitated in the final determination. All samples were prepared in fused silica or quartz beakers and quartz stirring rods were used. The solutions were kept in Pyrex glass containers. Blanks were determined on all chemicals used. The method was checked by the use of a standard sodium chloride solution.

Determination of Sodium in Wheat Products

A sample of wheat product containing not more than 20 to 25 mg. of sodium was weighed out in a platinum dish and ashed in the usual manner. The ash was transferred to a silica beaker, heated with 20 cc. of water and 5 cc. of 70 to 72% perchloric acid, and filtered. The filtrate was then evaporated to dryness and sodium and potassium precipitated by perchloric acid. $NaClO_4$ was separated from $KClO_4$ by dissolving in alcohol and filtering through a Bertrand filter. The filtrate was evaporated to dryness, taken up in water and several drops of phenolphthalein added. The solution was then made slightly alkaline with powdered calcium oxide. Enough calcium oxide was added so the solution remained pink after boiling for ten minutes, great care being taken to avoid an excess. The solution was allowed to stand overnight, filtered and washed with hot water. The filtrate was evaporated to a volume of 3 cc., and 30 cc. of freshly filtered sodium reagent added. The solution was kept in an ice bath for one hour or longer and stirred frequently. Precipitations done at room temperature gave irregular results. The solution was then filtered through a sintered glass crucible and the precipitate washed several times with 2 or 3 cc. of the reagent and finally two or three times with 2 cc. portions of 95% alcohol previously saturated with the triple salt. The precipitate was then dissolved in cold water and titrated with N/20 sodium hydroxide using phenolphthalein as an indicator. An excess of sodium hydroxide was run in and the solution heated to incipient boiling and kept there for five minutes. The red coloration should not disappear during heating. N/20 hydrochloric acid was used for back titration. The ratio of the base to the triple salt is 10:1. This method offered an advantage over the gravimetric procedure because the uranium content of the triple salt alone is titrated and therefore the degree of hydration of the triple salt does not enter the

calculation. The accuracy depends on the complete precipitation of the sodium and on the temperature at which this precipitation is made.

The problem of determining sodium on the product by a wet digestion process presented some difficulties. Any of the perchloric acid available contained high sodium blanks. A "vacuum-distilled" 70 to 72% perchloric acid put up in Pyrex was finally obtained for this purpose. This acid contained a small sodium blank. From 5 to 25 g. of product were digested with 25 cc. perchloric acid and 50 cc. concentrated nitric acid in the Vitreosil apparatus for five hours. At the end of five hours the sample was transferred to a Vitreosil beaker, evaporated to dryness on a hot plate, taken up in water and the sodium determined as outlined above.

Discussion

In Table I, the potassium content of wheat and its products are given together with the amount found in the ash. Potassium as determined directly on the product, varied from 0.109% in the patent flour to 1.539% in the bran. When the per cent of potassium in the product is calculated from the amount found in the ash, the amount of potassium varied from 0.100% in the patent flour to 1.501% in the bran. It will be seen from a comparison of these figures that a loss of from 2.4% to 8.2% occurred during ignition at 590° C. While this amount is small, it is too large to be due to experimental error.

TABLE I

POTASSIUM CONTENT OF HARD SPRING WHEAT AND ITS PRODUCTS
(RESULTS CALCULATED TO DRY BASIS)

Product	Ash	Potassium on ash	Potassium on product calculated to ash	Potassium on product	Potassium on ash calculated to product	Loss on ashing
	%	%	%	%	%	%
Patent	0.437	22.88	24.94	0.109	0.100	8.2
Clear	0.745	21.88	23.36	0.174	0.163	6.3
Wheat	1.989	24.41	26.14	0.520	0.485	6.6
Germ	4.499	24.42	25.58	1.151	1.099	4.5
Bran	6.652	22.56	23.14	1.539	1.501	2.4

In Table II, the sodium content of wheat and its products are given together with the amount found in the ash. Sodium, as determined directly on the product, varied from 0.001% in the patent to 0.005% in the bran. No loss of sodium was observed on ashing. High sodium results on the ash such as 9.55% sodium oxide, which are found in the literature, are doubtless erroneous. In none of the wheats examined

TABLE II

SODIUM CONTENT OF HARD SPRING WHEAT AND ITS PRODUCTS
(RESULTS CALCULATED TO DRY BASIS)

	Ash	Sodium on ash	Sodium on product	Sodium on ash calculated to product
	%	%	%	%
Patent	0.437	0.265	0.001	0.001
Clear	0.745	0.232	0.002	0.002
Wheat	1.989	0.152	0.003	0.003
Germ	4.499	0.119	0.004	0.005
Bran	6.652	0.077	0.005	0.005

have the authors found the true sodium content to be over 0.5% of the total ash.

Summary

The direct determinations for sodium and potassium on wheat and products milled from it are described in detail. These elements were also determined on the ash of wheat products.

A small loss of potassium was observed when ashing the samples at 590° C. for 16 hours, the largest loss being in the patent. No loss of sodium was observed on ashing.

The direct measurement of sodium by the method outlined in the preceding pages is believed to afford more reliable results than have hitherto been available on wheat and its products.

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SELENIUM IN PROTEINS FROM TOXIC FOODSTUFFS.[1]

I. REMARKS ON THE OCCURRENCE AND NATURE OF THE SELENIUM PRESENT IN A NUMBER OF FOODSTUFFS OR THEIR DERIVED PRODUCTS

KURT W. FRANKE and E. PAGE PAINTER

Department of Experiment Station Chemistry, South Dakota State College, Brookings, South Dakota

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A new toxicant carried in the protein fraction of certain grains was recently reported by Franke (1934, 1934a). Robinson (1933) reported the presence of selenium in wheat (No. 459 and others) which animal feeding had shown to be toxic (Franke, loc. cit.). The presence of selenium in affected foodstuffs is the only difference yet known between "toxic" and "normal" foodstuffs. Feeding trials using selenium salts (Franke and Potter, 1935) produced symptomatology almost identical with that produced by toxic foodstuffs. The pathology found is very similar to that described by Franke (1934). However, Byers (1934) reported the presence of vanadium in one sample of foodstuff obtained from the affected area. The maximum selenium content determined by available methods in the grains reported is approximately 30 p.p.m. The presence or absence of selenium which is reported in this paper is based on the results obtained by slight modifications of the method described by Horn (1934). The method used in this laboratory is as follows:

Method

From 1 to 5 g. of a sample is digested in a Kjeldahl flask with 35 to 70 cc. of concentrated sulfuric acid in the presence of a suitable catalyst (mercuric oxide) until it is colorless. (Slight yellow tinges may interfere with the test.) Two or three glass beads are added to the digesting mixture to prevent bumping. Ten cc. of this solution, after cooling, is poured into test tubes (Board of Health Tube) and 2 to 3 drops of saturated codein sulphate added. The tubes are shaken vigorously after adding each drop, then allowed to stand. A blue color indicates the presence of selenium, but it should be noted that vanadium also gives a blue color, as reported by Horn. The final volume of the digest must be considered when comparing the color developed. Comparisons should be made from three to twenty-four hours after the addition of the codein sulphate.

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[1] Published with the permission of the director of the South Dakota Agricultural Experiment Station as communication No.13 from the Department of Experiment Station Chemistry. These investigations are being carried out under the Purnell Fund and with the cooperation of the Bureau of Chemistry and Soils, Bureau of Plant Industry, Bureau of Animal Industry, and Bureau of Home Economics of the United States Department of Agriculture.

Occurrence of Selenium in Cereals

This rapidly performed test permitted a large number of tests for selenium to be made on grain samples, their components, and other biological material. The results obtained in this laboratory using the alkaloidal test are thus briefly summarized: Every sample known to be toxic gave a definite positive test. All samples obtained from areas remotely separated from the affected areas, and known to be "normal," gave negative tests. A large number of samples of questionable toxicity gave faintly positive or questionable tests. All these were obtained from suspected affected areas. Tests were made on 48 grain samples, including wheat, corn, barley, oats, emmer, and rye, all of which had been previously bio-assayed by feeding trials (Franke, 1934) and differentiated as "lethal," "sub-lethal," "questionable," and "normal" grains. The toxicity of the different grain samples, however, did not vary with the selenium content as indicated by the codein-sulfate test. In samples of the same kind of grain there appeared to be some correlation with the results obtained by feeding trials. No attempt has been made to estimate the amount of selenium present by the intensity of the blue color developed. The results do not preclude the possibility that in some cases part of the blue color produced is due to vanadium. A positive test could be obtained on as little as 0.5 g. of "lethal" wheat.

The selenium present in toxic grain samples is probably all in the protein fraction. Franke and Moxon (1934) reported that the protein from toxic grains will not stimulate carbon dioxide production by yeast cells. Selenium was found in the proteins of corn, wheat, barley, and in many animal proteins in cases where the animals had been fed toxic foodstuffs. Positive tests were obtained on eggs, egg albumin, vitelline, blood plasma, red cells, liver, spleen, kidneys, lungs, and also on the excreta. No protein from a "normal" foodstuff has given a positive test. Dudley and Byers (1935) have also reported the presence of selenium in various animal products.

Probable Nature of Selenium

It is generally considered that metals in the ionic state are always more toxic than when in the metallic state. However, it seemed desirable to determine whether or not the selenium was adsorbed on the protein molecule.

The selenium in the proteins is not removed by any solvent yet tried. The selenium in the samples obtained by this laboratory differs from that reported by Beath *et al.* (1934) who found the selenium present in water soluble compounds.

In the following experiments gluten from toxic wheat (Laboratory No. 582) was used as this is probably a typical toxic protein. The experimental work reported by Franke and Potter (1934) and Franke and Moxon (1934) was based on this sample. The protein used in these investigations was kneaded from finely ground wheat and reprecipitated from 0.075 N acetic acid by salting out, using sodium chloride, following the method described by Blish and Sandstedt (1926). Although some of the proteins were lost in these separations, tests indicated that the selenium content of these was the same as that of the recovered proteins.

The common solvents for metallic selenium are concentrated sulfuric acid, nitric acid, carbon disulphide, potassium cyanide, and free halogens. The first two strong acids could not be used due to their destruction of the protein.

By shaking a solution of toxic protein peptised in acetic acid with carbon disulfide, no selenium was removed.

When the protein was peptised by potassium cyanide, placed in a cellophane bag immersed in water, no selenium dialized out. By similarly treating metallic selenium, it readily dializes out; the potassium selenocyanide, which is formed, readily passes through the membrane.

A solution of bromine in hydrobromic acid was added to a protein "solution" in dilute acetic acid. This was electrodialized until the protein coagulated. A very small amount of selenium was found in the cathodic cell. Undoubtedly, some protein hydrolysis occurred. Whether the selenium was actually split off and transported as the positive selenium ion (when selenium halides are formed) or whether it was in an organic compound resulting from hydrolysis is not known. In either case, it would move toward the cathode. In such a strongly acid solution, organic compounds which ionize, behave as bases, because their iso-electric points are nearer neutral than the pH of mineral acid solutions. It was impossible to remove more than a trace of selenium in this manner. No diminution of the selenium content of the protein could be observed. Electrodialysis of protein "dissolved" in .075 N acetic acid failed to remove any selenium.

When a protein peptised in 0.2 N sodium hydroxide was electrodialized, a small amount of selenium appeared in the anodic cell, but it was not possible to appreciably reduce the selenium content of the protein by this procedure. It seemed likely that some of the selenium was in a very labile form (similar to sulphur in proteins) readily split from its former linkage by alkalies. There is also the possibility that a selective hydrolysis has removed the compounds containing the selenium.

After these attempts to remove selenium from proteins failed, investigations were begun upon the products of protein hydrolysis. When toxic proteins were hydrolized with acids, most of the selenium remained in solution, but some was in the humin. Electrodialysis of a neutralized hydrolysate failed to separate the selenium from the known products of hydrolysis. Since the selenium did not possess the electrolytic properties of an inorganic compound, there remains little doubt that it is in some organic compound or compounds.

Conclusions

The codein-sulfate test for selenium has been found to be reliable as a negative test.

Selenium in affected foodstuffs is present in the protein fraction.

Little, if any, selenium is present as metallic selenium or in an inorganic salt.

When toxic proteins are hydrolized, most of the selenium remains in solution in some organic compound or compounds.

Some selenium appears to be in a very labile form, readily split by alkalies.

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THE INFLUENCE OF VARIOUS FACTORS INCLUDING ALTITUDE ON THE PRODUCTION OF ANGEL FOOD CAKE

MARK A. BARMORE

Colorado Agricultural Experiment Station, Fort Collins, Colorado

(Read at the Annual Meeting, June 1935)

The main problem with which the Home Economics Section of the Colorado Experiment Station is concerned, is that of the baking of flour mixtures at high altitudes. The effects of high altitude are such that no successful sea-level cake recipe can be used at altitudes above three to four thousand feet. The higher the altitude the more impossible these recipes become.

To give some idea of the area of the United States involved in the altitude baking problem, the following map, Figure 1, is presented.

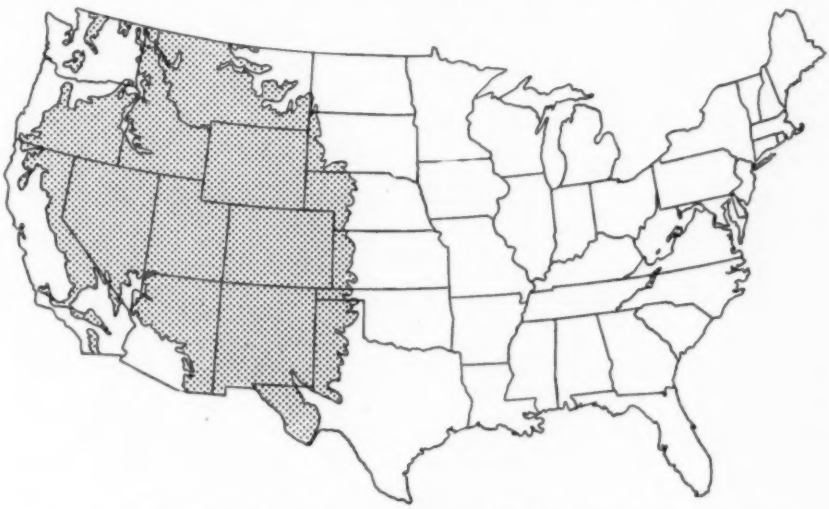

Fig. 1. Map of the United States showing that portion (the shaded area) lying 3,000 feet or more above sea level.

This high altitude region comprises slightly more than one-third of the United States, and although this area is sparsely populated, about five million people make their homes here, according to the 1930 census.

In order to study this problem more intelligently, equipment designated as the altitude laboratory, was constructed and installed.[1]

[1] Mr. J. Harry Scofield, Associate Professor of Mechanical Engineering, Colorado State College, designed and supervised the construction and installation of this equipment.

Figure 2 shows schematically the type and arrangement of the installation. The laboratory itself is a steel cylinder 7 feet in diameter and 9 feet high. The equipment is so designed that this laboratory can be ventilated, the temperature and humidity controlled, and the air pressure adjusted and maintained at a value corresponding to any altitude in the range from sea level to 18,000 feet above sea level.

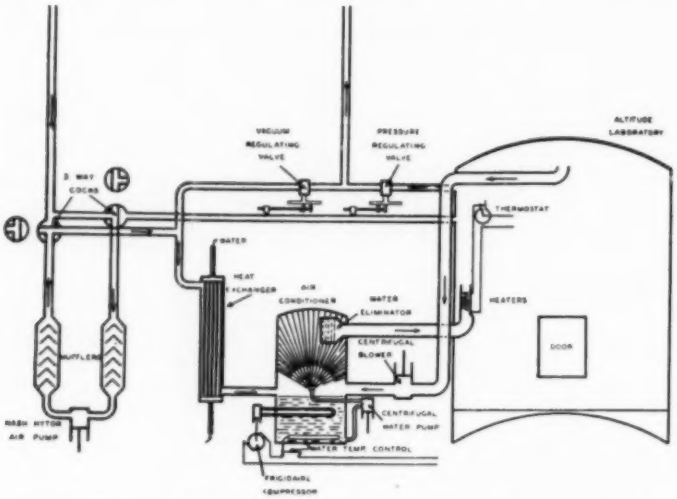

Fig. 2. A schematic drawing showing the various parts of the apparatus.

Whitacre (1922), McKittrick (1923), Fredrickson and Dozier (1928), Steckelberg (1928), and Peterson (1930) have devised recipes for various altitudes at Fort Collins and elsewhere. These workers made no attempt to investigate fundamental effects of altitude and other factors. The intention in this study was to investigate the problem in as fundamental a manner as possible, with the expectation that in the long run more practical results would be obtained and much more would be learned about baking in general.

The first step was to investigate the effects of altitude on the baking process. The simplest type of flour mixture was chosen and a standard method of preparation worked out. The chosen mixture is known as angel food cake, and consists of egg white, flour, sugar, and a small amount of acid or acid salt.

By means of thermocouples, buried in the mixture, the temperature change within the cake was followed as the baking progressed as well as during the cooling period.

Figure 4 shows the effect of the change in altitude on the internal temperature of cakes baked at 165° C. at sea level, 5,000 and 10,000 feet of altitude. The maximum temperature was about that of the

boiling point of pure water at each altitude chosen, although the moisture was present as a concentrated solution. No consistent change could be found in the rate of temperature rise during the first part of the baking period at the different altitudes.

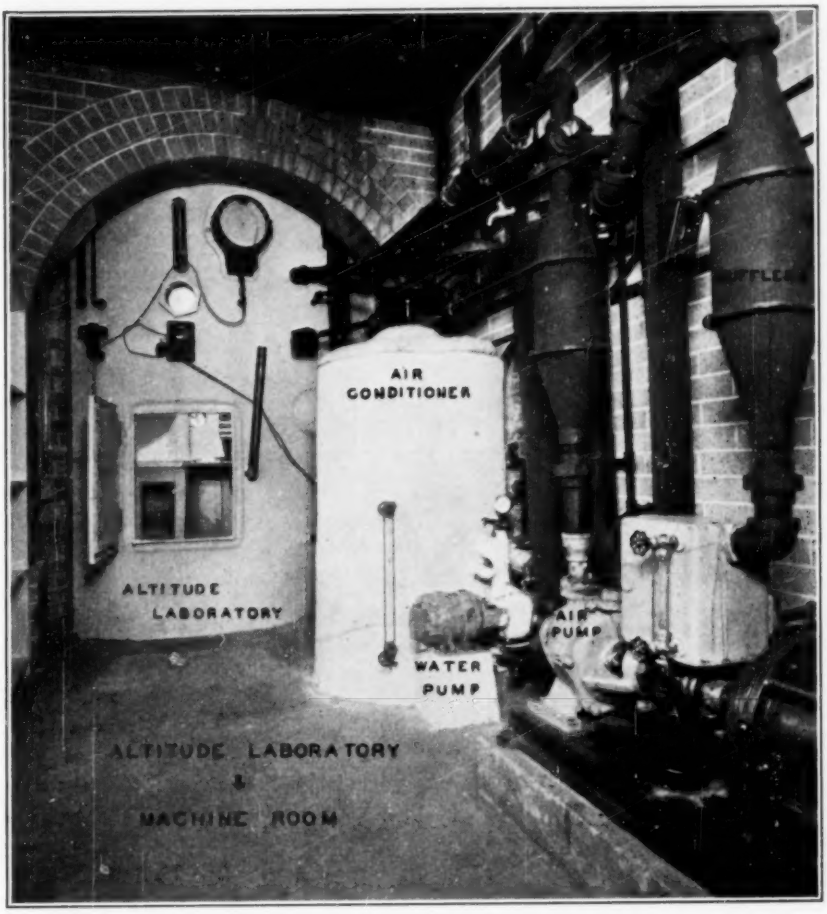

Fig. 3. A view taken from the door of the machine room.

The rate and amount of evaporation during baking were determined by suspending the cake inside the oven from a balance on top of the oven. The rate of evaporation, and the effect of changes in altitude on the rate of evaporation from cake batter weighing 380 grams, baked for 45 minutes, at 165° C., is shown in Figure 5. Although it is not apparent in the figure, the slopes of the straight portions of the curves are slightly greater at the higher altitudes. The averages obtained were 0.99, 1.06, and 1.15 grams per minute.

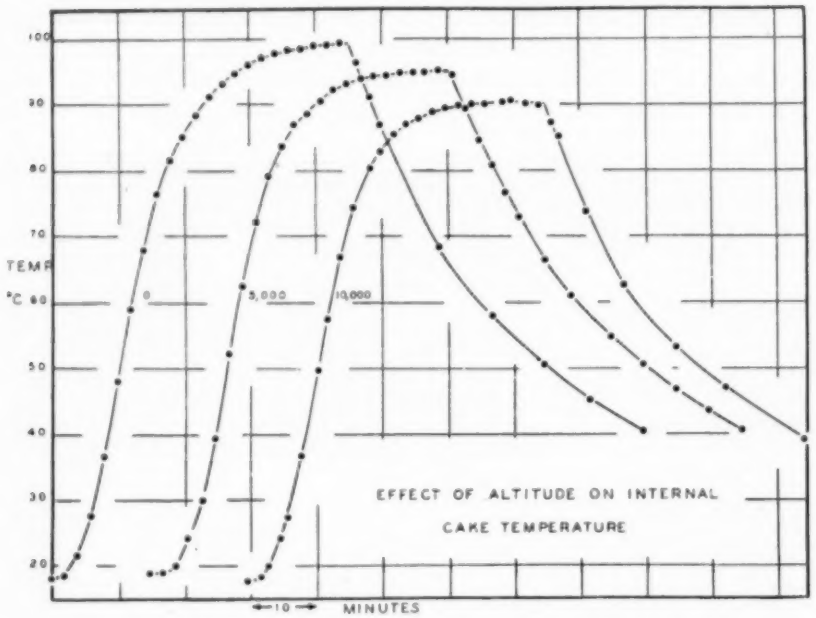

Fig. 4.

This difference is easily explained by the fact, shown in the Figure 4, that the temperature difference between the oven and the cake increased as the altitude increased, thus allowing more heat to be carried into the cake which must in turn be dissipated by evaporation.

The batters expanded, or raised, more during baking at high altitudes than at low altitudes. This increased expansion at higher

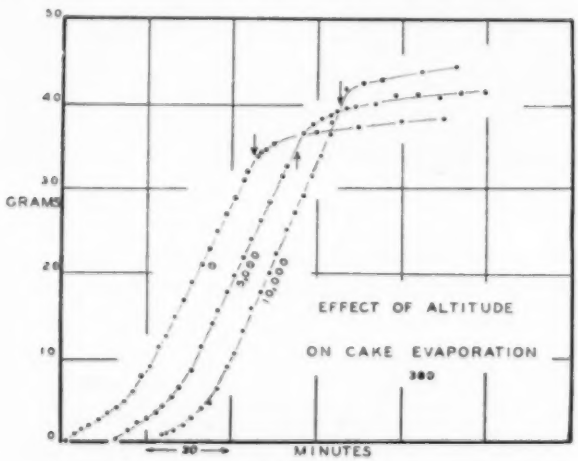

Fig. 5.

altitudes was apparently due to the increased volume of water vapor which was given off. Assuming that the data given on internal cake temperature and rate of evaporation applies, the volume of water vapor escaping at the different altitudes was found to be as follows:

Altitude	Volume Liters per minute
0	1.65
5,000	2.11
10,000	2.70

There is considerable reason to believe that the absolute amounts of these figures are very misleading, because it has been possible to show that probably 95% of the moisture lost during baking comes from that portion of the cake lying within one centimeter of the crust. However, it seems reasonable that the increased cake expansion, as the altitude was increased, was due to an increased volume of water vapor liberated, and, that this volume of vapor increased in the same relative manner, although the amounts were only a small fraction of the previously given figures.

Increases in altitude were found to decrease the amount of browning or caramelization taking place in the crust, and was due to a decreased crust temperature.

The crude piece of apparatus, shown in Figure 6, was copied from work done by Platt and Kratz (1933), and was used to measure the tensile strength or tenderness of the cake samples. It consists merely of two clamps attached to the specimen, which was cut from the cake in a standard manner. A small bucket was hung on the lower clamp, into which water was run at a constant rate until the sample broke. The total weight necessary to pull apart a section of cake one square centimeter in cross-sectional area was taken as the value for tensile strength.

It has been possible to show by means of this test that the tensile strength decreases with altitude according to the following linear equation:

$$T = -0.82\,A + 20.2,$$

where T and A represent tensile strength in grams per sq. cm. and altitude in thousands of feet.

It is believed that this change in strength is due to the combined effect of the increased expansion and the decreased internal cake temperature encountered at high altitudes. It is thought that the increased expansion stretches the structure beyond its elastic limit and that the reduced temperature changes the balance between water,

starch, protein, and sugar sufficiently to alter their physical properties. The result, unless the proportion of ingredients is changed, is a structure too weak to support itself and therefore is a failure from a culinary standpoint.

By this method it has been possible to show that a high baking temperature does not decrease the tenderness, as has been claimed by some. This is doubtless due to the fact that the internal cake temperature was changed but little for a relatively large change in oven

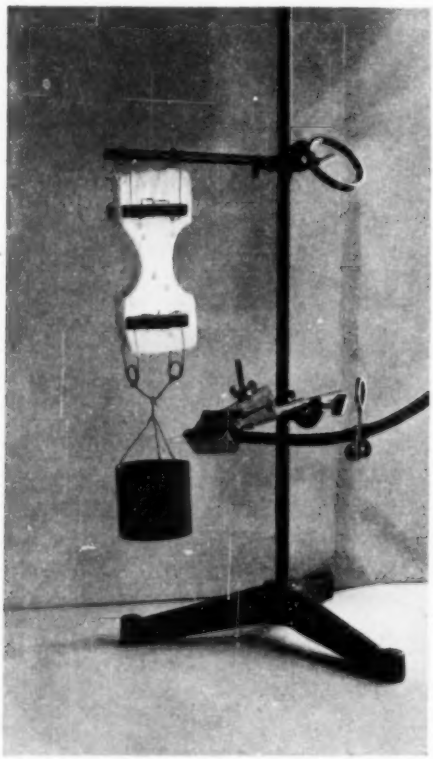

Fig. 6. The tensile strength testing apparatus.

temperature (Fig. 7). The temperatures were measured by means of thermocouples and the oven temperatures are shown beside the curves. It will be noticed that a difference of 42° C. in oven temperature produced a drop of only about 2° C. in maximum temperature.

On investigating the effects of ingredients it was found that increases in egg white and flour increased the tensile strength. The logical thing to do, to correct the recipe for changes in altitude, was then to increase the flour or egg white or to decrease the sugar content.

But the question was how much increase or decrease was required. It was found that if the amount of egg white was allowed to remain constant at 210 grams the influence of alterations in the amounts of other ingredients produced a change in the tensile strength of the cake which was a linear function of the change in the ingredient. This

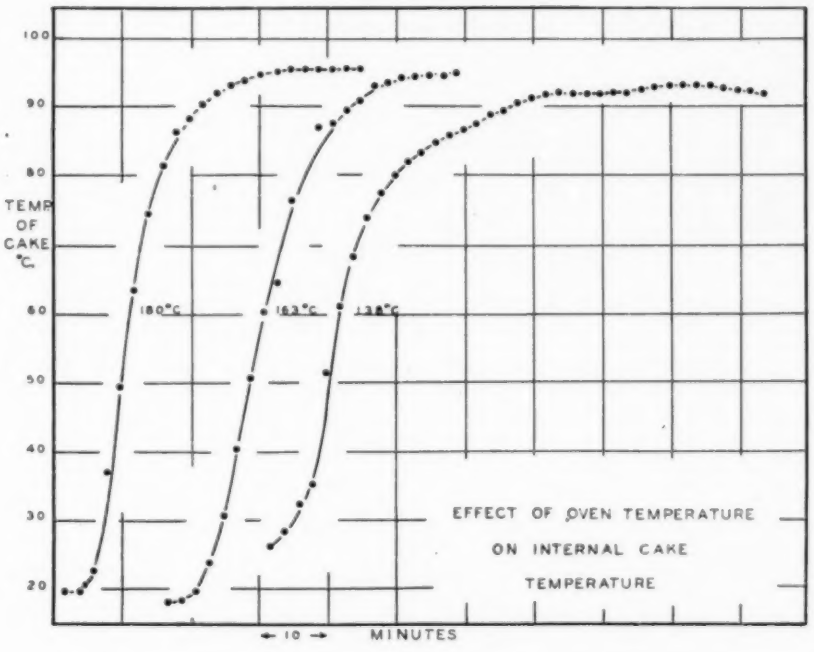

Fig. 7.

resulted in the following linear equations where F and S are the flour and sugar content in grams and T is the tensile strength in grams per square centimeter.

$$T = \quad 0.20\,F + \quad 5.0,$$
$$T = -\,0.085\,S + 29.5.$$

These two equations and the one for the effect of altitude when combined gave an equation of this order

$$T = 0.20\,F - 0.085\,S - 0.82\,A + 21.9.$$

This equation was found to be approximately correct, but, since there is no practical need to produce cakes of varying tensile strength, the equation is of no practical use as it stands. From a culinary standpoint, cakes should have as low a value for tensile strength as possible to produce successful cakes. The reason for this is due to the

application of the absurdly simple rule "that cakes which fall are not strong enough to stand" or the tensile strength is not great enough. Thus it is necessary to know the tensile strength of cakes just barely strong enough to stand. For angel food cake this value was found to be about 17 grams per sq. cm., which was then substituted for T in the equation and incorporated into the constant yielding the equation

$$F - .43\,S - 4.1\,A + 24.5 = 0.$$

This expresses all of the possible, successful recipes for this type of flour mixture for any habitable altitude. There are of course limits in the amounts of ingredients outside of which it is not practical to go (F should not be less than 40 nor more than 80 grams). No successful recipes were found which disagreed. A large number of the various combinations were tried and they were entirely successful in every case.

This study has yielded not only recipes for high altitudes but for low altitudes and sea level as well. It explains very vividly what is meant by balancing of baking formulae. It explains, at least in part, some of the effects of altitude on baking. It points the way for further investigations on the more complex types of flour mixtures. But most important of all it shows that these complex mixtures, that have previously been devised by hit and miss methods, yield to investigation and show that they conform to a systematic scheme.

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EFFECT OF HARVEST CONDITIONS ON A FEW QUALITY FACTORS IN WHEAT[1]

C. O. SWANSON

Kansas Agricultural Experiment Station, Manhattan, Kansas

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Introduction

The most important harvest conditions are those related to moisture and temperature from the time the wheat kernel is fully developed until it is placed in the storage bin. Wheat kernels ripe enough to be cut with a binder and cured in the shock may have from 30 to 40% of moisture. Wheat cut with the combine harvester and thresher should preferably have less than 14% of moisture. If the moisture in combined wheat is higher than this there is danger of heating due to the respiration of the wheat itself and the respiration of molds growing on the wheat.

The fact that wheat is biologically active is the main reason the harvest conditions of moisture and temperature affect the quality of wheat. This activity responds to irritation or stimulation by moisture and temperature as other living organisms. In these experiments most attention was given to moisture although temperature was also considered.

Previous Experiments

RESPIRATION

One of the best known manifestations of biological activity is respiration. The rate of respiration in wheat is usually determined by estimating the milligrams of carbon dioxide evolved in 24 hours per 100 grams of wheat. Wheat ripe enough to cut with a binder and which may contain about 30% moisture is very much more biologically active than wheat with the moisture reduced to the percentage necessary for harvesting with the combine (see Swanson and Fenton, 1930). Whether the moisture is inherent or added by rain seems to make little difference. It is rather the amount of moisture (Swanson, 1934) which determines the rate of biological activity.

The rate of respiration was found by Bailey and Gurjar (1918) to be about twice as great at 15% moisture as at 14%, and again from two to nearly four times as great at 16 as at 15%. Immature shrivelled

[1] Contribution No. 50, Department of Milling Industry.

wheat was found to have approximately twice the respiration rate of plump mature wheat of equal moisture content. Thus the life history of the kernel influences the rate of biological activity aside from the moisture content. That is, a kernel whose growth activities were stopped by cutting before it was fully matured responds to the stimulation of moisture at a greater rate than a kernel which has been completely matured.

Diastatic Activity

In using the term "diastatic activity" a distinction should be made between the sugar present in the kernel while still intact, and the sugar produced when wheat meal is digested in water for a definite time and temperature. (See Blish and Sandstedt, 1933.) The latter is the measure of diastatic activity proper. The sugar found in the kernel before crushing may be either that which was left after the starch synthesizing processes were stopped during desiccation, or it may have been produced by the activity of the diastatic enzymes of secretion which are derived from the epithelial layer of cells between the germ and the endosperm. These enzymes are activated when enough moisture is present to start the process of germination (Swanson, 1935). Thus the sugar which is present before digesting meal or flour from sound wheat is either that which was left or laid down in the endosperm during its formation, or additional amounts which may have been produced by moisture conditions after the wheat was cut. An increase in sugar due to the stimulation of moisture is accompanied by an increase in diastatic activity. That is, when meal from such wheat is digested in water more sugar is produced than from wheat which has not been subjected to high moisture. It apparently requires more moisture to increase the sugar content than to increase the rate of diastatic activity. One reason may be that when the amounts of moisture are only enough to increase the rate of respiration but not enough to start the process of germination, the increased rate of respiration consumes the sugar as fast or faster than it is produced.

Amounts of wetting such as would be produced by small rains were shown to be without effect either in increasing the sugar content of wheat or in affecting its diastatic activity (Swanson, 1935). Small rains however may have an influence on other factors which are used to measure quality in wheat. The color of the bran coat might be bleached and the test weight decreased because of the roughening of the bran surface and the swelling of the kernel. Better baking behavior is sometimes obtained from wheat which has been exposed to a moderate amount of weathering than from wheat not so exposed.

It may be that the activity of enzymes associated with the post-harvest maturing process has been stimulated, but effects on the diastases have not been observed.

Experiments in 1934

In the summer of 1934 experiments on the effects of various harvest conditions on some of the quality characteristics of wheat were further pursued. There were included in these studies not only the effects of wetting, but also the effects of harvesting at various stages of ripening. For convenience in presentation the two sets of conditions will be grouped under separate headings, as follows:

PART I. The effect of cutting wheat at five stages of ripening and then drying four separate portions from each stage under four conditions: (1) in the sun; (2) in the sun but covered with paper; (3) in a basement room; and (4) in a room kept at about 55° F.

PART II. The effect of wetting wheat to various degrees after it was cut. One part was cut at the maturity suitable for the binder, and the other part at the dryness required for the combine. Half of each portion of these samples was dried in the sun after wetting and half in a shed.

Part I

Effects of Cutting at Various Stages of Maturity

The work done on wheat cut at the five stages of maturity will be considered first. The aim was to cut the wheat when the endosperm was in one of the following conditions: (1) milk, (2) soft dough, (3) hard dough, (4) tough, and (5) hard. Determinations on separate samples threshed at the time of cutting gave the following moisture percentages: (1) 43.5, (2) 41.3, (3) 35.3, (4) 29.0, and (5) 13.0. The dates of cutting were: (1) June 4, (2) June 6, (3) June 8, (4) June 9, and (5) June 13. The last two stages coincided with the time and conditions for cutting the samples used in Part II.

Four Conditions of Drying

The four conditions of drying the wheat cut at various stages of maturity were designed to imitate: (1) ordinary harvesting, by drying in the sun; (2) nursery harvesting, by covering with paper and placing in the sun; (3) drying in cloudy but warm weather, by placing in the cool basement; (4) drying during rather cool and cloudy weather, by placing in the cold room at from 50 to 60° F. There were four bundles from each stage of cutting and hence the four conditions of drying were used for each stage. The basement room was under a large stone building, and the cold room was the cheese room of the Dairy Depart-

ment. The relative humidity in this room ranged from 60 to 70 and the temperature from 50 to 55° F. The bundles for this room were placed in cloth sacks to avoid littering. Because of this covering the rate of drying was somewhat retarded.

After all the various bundles were dry they were threshed on the nursery thresher and the wheat was subjected to various tests designed to measure the effects on quality.

Test Weight as Influenced by Stage of Cutting and Conditions of Drying

Test weights were taken using the half pint kettle both after the samples had been passed through the small laboratory grain cleaner and after the samples had been scoured on the small scourer. The cleaner did not in all cases remove the white caps. The scourer not only removed these but also left the bran coats in a smooth condition which would facilitate closer packing. The figures for test weights are given in Table I. Cutting the wheat at moisture contents of

TABLE I

Test Weight as Influenced by Stage of Cutting and Conditions of Drying

Moisture when cut	Conditions of drying							
	Sun		Covered		Basement		Cold room	
	Cleaned	Scoured	Cleaned	Scoured	Cleaned	Scoured	Cleaned	Scoured
%	*Lbs.*	*Lbs.*	*Lbs.*	*Lbs.*	*Lbs.*	*Lbs.*	*Lbs.*	*Lbs.*
43.6	59.5	62.0	59.0	61.5	62[1]	62.5	58.0	60.5
41.3	59.5	62.0	59.5	62.0	59.0	61.5	58.0	61.0
35.3	59.0	62.0	59.8	62.5	59.5	62.0	58.5	61.5
29.0	60.9	63.5	61.0	63.5	60.5	63.5	60.5	63.5
13.0	60.0	63.0	60.0	62.5	59.6	62.0	59.0	62.0

[1] Scoured.

35.3% or more decreased the test weight in all cases. The decreases however seem small when the high moisture content is considered, but it would be enough to lower the market grade at least one point. The differences in test weight as affected by drying under the three conditions—sun, covered, or in the basement—were within experimental error, but drying in the cold room decreased the test weight of the samples cut at moisture contents of 35.3% or above. When wheat was cut at 29% and 13% moisture the influence of the low temperature was negligible. The scouring increased the test weight in all cases.

Grade and Percentage of Yellow Berry as Influenced by Stage of Cutting and Conditions of Drying

These wheat samples were studied for market grade by Professors J. W. Zahnley and C. D. Davis. This study revealed that the numerical grades would be determined by the test weight and the subclass by the percentage of yellow berry. This percentage was determined by actual separation of yellow and vitreous kernels in 10-gram samples. The figures for percentage yellow berry kernels are given in Table II.

TABLE II

Percentage of Yellow Berry as Influenced by Stage of Cutting and Condition of Drying

Moisture when cut	Conditions of drying			
	Sun	Covered	Basement	Cold room
%	*Per cent yellow berry*			
43.6	19.1	24.0	24.5	21.2
41.3	27.3	28.1	31.7	18.4
35.3	14.2	15.6	17.6	25.9
29.0	21.8	25.8	33.7	26.3
13.0	61.4	51.7	67.9	74.0

The percentage of vitreous kernels would be the differences of these figures and 100. These figures show that the condition of drying had no consistent effect on the percentage of yellow berry kernels, neither did the stage of cutting show any effect except the last which had a considerably higher percentage of yellow kernels. Whether this was due to letting the desiccation proceed further, or whether it was due to some field condition was not learned.

Sugar Content and Diastatic Activity as Influenced by State of Cutting and Conditions of Drying

The method of determining sugar and diastatic activity was that of Blish and Sandstedt (1933). The data obtained are given in Table III. The figures under sugar mean the milligrams maltose in 10 grams wheat meal present in soluble form before digestion, and the figures under diastatic activity mean the total milligrams maltose obtained after the 1 hour digestion. The differences between these two sets of figures would be the amount of maltose produced by the saccharogenic activity during the 1 hour digestion.

The sugar contents show no variations which may be due to stage of cutting or condition of drying, except perhaps that the samples cut at the two higher stages of moisture and dried in the basement and

TABLE III

THE SUGAR CONTENT AND DIASTATIC ACTIVITY AS INFLUENCED BY STAGE OF CUTTING AND CONDITION OF DRYING

Moisture when cut	Sugar content [1]				Diastatic activity [2]			
	Sun	Covered	Basement	Cold room	Sun	Covered	Basement	Cold room
%	*Mg.*	*Mg.*	*Mg.*	*Mg.*	*Mg.*	*Mg.*	*Mg.*	*Mg.*
43.6	55	55	45	40	340	326	319	505
41.3	60	53	45	40	298	292	272	420
35.3	55	53	50	55	278	278	306	674
29.0	55	55	55	50	340	330	330	602
13.0	50	50	48	50	246	246	282	280

[1] Mg. maltose per 10 g. wheat meal before digestion.
[2] Mg. maltose per 10 g. wheat meal after digestion for 1 hour at 30° C.

cold room had a lower sugar content. This may have been due to consumption of sugar by respiration which persisted longer and at a higher rate in these samples.

The lowest diastatic activity was obtained in the wheat samples cut at 13% moisture or the combine stage. Drying in the sun, covered, or in the basement produced no notable differences in diastatic activity. Drying in the cold room did, however, produce a very notable increase in diastatic activity.

Thus the temperature at which the wheat is dried when cut at higher moisture contents seems to have a pronounced influence on diastatic activity. This effect of temperature in influencing diastatic activity may be a partial explanation for the higher diastatic values often obtained from spring wheats as compared with winter wheats. The temperatures are prevailingly lower during harvest in the spring wheat area than in the winter wheat area. Temperatures like 50 to 60° F. which had a marked effect would not be uncommon in the spring wheat territory, especially in Western Canada. The effects of moisture will be discussed later. There are thus apparently two reasons for the low diastatic activity in much of the hard winter wheat: the low moisture content of the combined wheat and the high temperature prevailing during harvest.

Part II

THE EFFECT OF WETTING WHEAT TO VARYING EXTENTS

Half of the samples of wheat used for these trials were cut at 29% moisture or when ripe enough for the binder-harvester, and half at 13% moisture or when dry enough for the combine-harvester. The treatments in respect to the wetting and drying of the wheat after cutting

were the same for each of the two stages. There were two conditions of drying after wetting, one outdoors in the sun, and the other in a well ventilated shed to imitate drying in cloudy weather. The wetting was done by placing the bundles heads down in tall tanks filled with water to such an extent that the head half of the bundles was immersed. The time of soaking for one-half of all the bundles was 10 minutes, and for the other half it was 30 minutes. The number of wettings and dryings for different bundles was varied from one to four, *i.e.*, some bundles were wetted but once, others 2, 3, and 4 times. Besides these were the checks which were not wetted, but kept either in the sun or shade with the others.

Test Weight as Affected by Extent of Wetting and Conditions of Drying

The test weights were obtained on the cleaned wheats but the cleaning did not remove the white caps from all samples. The presence of this would lower the test weight somewhat. The figures obtained are given in Table IV. These samples were also judged for market

TABLE IV

Test Weight as Affected by Amount of Wetting and Conditions of Drying

Moisture when cut	Place dried	Time soaked	Times wetted				
			0	1	2	3	4
%		*Min.*	*Lbs.*	*Lbs.*	*Lbs.*	*Lbs.*	*Lbs.*
29	Sun	10	59.1	60.5	61.0	60.1	59.4
29	Shade	10	60.6	61.1	60.4	56.8[1]	61.0
13	Sun	10	60.0	59.2	59.0	59.5	59.7
13	Shade	10	60.4	58.6[4]	58.5	57.6[3]	57.3[3]
29	Sun	30	60.0	60.8	60.3	59.8	59.2
29	Shade	30	56.5[1]	60.0	59.8	57.5[2]	57.9[2]
13	Sun	30	60.0	59.8	59.6	59.8	59.1
13	Shade	30	59.7	58.8	58.1[4]	58.4	57.7[4]

[1] Contained white caps and was musty.
[2] Sprouted and weathered.
[3] Damaged kernels.
[4] Dull color, no luster.

grade by Professors Zahnley and Davis. Wherever a notable lowering in test weight occurred it was accompanied by a distinct visible damage such as can be detected by inspection in grain grading. The amount of wetting had less effect than the conditions of drying. None of the samples dried in the sun had suffered any distinct damage to the grading factors. The samples placed in the shade where the time of drying was prolonged were damaged because such conditions produced sprouting or mustiness.

Percentage of Yellow Berry and Apparent Damage as Influenced by Amount of Wetting and Drying

The figures for the percentage of yellow berry in the samples subjected to different amounts of wetting and drying are given in Table V.

TABLE V

Percentage of Yellow Berry and Apparent Damage as Influenced by Amount of Wetting and Drying

Moisture when cut	Place dried	Time soaked	Times wetted				
			0	1	2	3	4
%		*Min.*	*Per cent yellow berry*				
29	Sun	10	63.1[1]	29.0[1]	25.5[1]	37.6[1]	69.4[1]
29	Shade	10	45.8[1]	29.8[1]	36.9[1]	63.6[3]	37.5[3]
13	Sun	10	72.7[1]	76.4[1]	79.0[1]	60.7[1]	79.3[1]
13	Shade	10	69.9[1]	88.2[1]	83.8[2]	78.2[3]	82.2[3]
29	Sun	30	44.6[1]	15.6[1]	52.1[1]	26.6[1]	47.2[1]
29	Shade	30	47.5[2]	39.9[2]	66.0[2]	35.7[4]	65.9[4]
13	Sun	30	51.9[1]	57.6[1]	65.8[1]	61.0[2]	74.2[2]
13	Shade	30	61.8[1]	24.2[1]	78.3[2]	92.7[3]	88.9[3]

[1] Bright, good luster.
[2] Bright.
[3] Discolored and musty.
[4] Sprouted.

The percentage of yellow berry was notably greater in the samples cut at 13% moisture than in those cut at 29%. The lack of correlation between the amount of wetting and the percentage of yellow berry indicates that the amount of yellow berry was determined by conditions existing before the wheat was cut, and that repeated wettings and dryings had but little if any influence. Repeated wettings followed by dryings, however, had a tendency to discolor and produce mustiness, and in a few cases sprouting. These effects were produced much more when the drying took place in the shade than in the sun. This indicates that small rains, such as would be represented by the 10-minute soakings, followed by sunshine, have apparently no effect on the appearance. Even two 30-minute soakings followed by drying in the sun had no effect. However, repeated wettings with rains which are followed by cloudy weather, such as would be represented by the 3 or 4 soakings and drying in the shade, may produce discoloring and sometimes mustiness or even sprouting.

Sugar Content and Diastatic Activity as Influenced by the Extent of Wetting and Condition of Drying

The figures for milligrams maltose per 10 grams wheat meal, or sugar content before digestion, and the milligrams maltose produced

during the 1 hour of digestion indicating amount of diastatic activity, are given in Table VI. The number of soakings and the drying in the

TABLE VI

SUGAR CONTENT AND DIASTATIC ACTIVITY AS INFLUENCED BY AMOUNT OF WETTING AND CONDITION OF DRYING

Treatment			Sugar content [1]					Diastatic activity [2]				
Moisture when cut	Place dried	Soaked	Times wetted									
			0	1	2	3	4	0	1	2	3	4
%		*Min.*	*Mg.*	*Mg.*	*Mg.*	*Mg.*	*Mg.*	*Mg.*	*Mg.*	*Mg.*	*Mg.*	*Mg.*
29	Sun	10	50	54	52	48	48	278	306	318	291	304
13	Sun	10	57	56	52	54	56	280	270	266	272	260
29	Shade	10	50	47	60	50	52	260	322	287	312	405
13	Shade	10	54	46	42	38	42	270	262	264	276	322
29	Sun	30	48	56	47	50	50	281	305	318	301	286
13	Sun	30	56	50	50	62	52	262	259	250	281	266
29	Shade	30	60	54	46	46	48	288	306	465	456	550
13	Shade	30	52	46	40	44	38	302	256	290	304	475

[1] Mg. maltose per 10 g. of wheat meal.
[2] Mg. maltose per 10 g. of wheat meal after 1 hour digestion at 30° C.

shade as compared with the sun had no consistent effect on sugar content either when the length of the soaking period was 10 minutes or 30 minutes.

The diastatic activity was not notably influenced by the 10-minute wetting periods except one wetted four times and dried in the shade. This sample was musty, *cf.* Table V. When cut at 29% moisture, wetted four times and dried in the shade there was an increase in diastatic activity starting with the second wetting period. Thus the length of time that the wheat remains wet is a factor which is important in stimulating diastatic activity.

These results indicate that even fairly heavy rains followed by sunshine or rapid drying have little or no influence in developing diastatic activity. However, when rains are followed by cloudy weather, they have an influence. The samples which showed an increase in diastatic activity were also the samples which showed apparent damage. Thus to increase diastatic activity requires considerable wetting and a duration of the wet condition so as to start the process of germination or induce conditions which cause damage.

DIASTATIC ACTIVITY OF THE BRUSH AND GERM ENDS OF THE WHEAT KERNEL

A few of the samples from these experiments were separated into germ and brush ends, and the diastatic activity determined on these

respective portions. Some samples were selected which had high and some which had average diastatic activity. The diastatic activity was also redetermined on the whole wheat kernels. The data obtained are found in Table VII.

TABLE VII

DIASTATIC ACTIVITY OF GERM AND BRUSH ENDS COMPARED WITH WHOLE WHEAT

Moisture when cut	Treatment	Milligrams maltose [1]		
		Whole kernels	Germ end	Brush end
%				
35.3	Dried in sun	289	272	258
35.3	Dried in cold room	612	520	565
29.0	Dried in sun	343	335	310
29.0	Dried in cold	481	390	370
13.0	Dried in sun	246	264	240
13.0	Dried in cold	257	268	244
29.0	No wetting, dried in shade	276	288	258
29.0	Wetted four times, 30 minutes each, dried in shade	548	430	465
13.0	No wetting, dried in shade	288	284	262
13.0	Wetted four times, 30 minutes each, dried in shade	427	378	288

[1] After 1 hour digestion at 30° C.

The differences between the brush ends and the germ ends were not large. In three of the 10 samples the brush gave a higher figure than the germ end. The germ end in most samples was lower than the whole kernel. The diastatic activity of the whole kernels was, in some cases, lower than shown for the same sample in Tables III and VI. This may have been due to the samples standing in the warm laboratory for some time after the first determination. This may also explain why the germ and brush ends averaged lower than the whole kernels.

SUGAR CONTENT AND DIASTATIC ACTIVITY OF BRUSH AND GERM ENDS AS INFLUENCED BY CONDITIONS OF GERMINATION

After separating a few typical samples into the brush and germ ends respectively, these together with the whole wheat were placed in the germinator of the seed laboratory for 72 hours. No counts were made but the sprouting was pronounced on the whole wheat and the germ ends. The wheat material was removed from the wet blotters and placed in shallow pans and allowed to become thoroughly air dry. After this the samples were ground and the sugar content as well as the diastatic activity determined. The data obtained are found in Table VIII.

TABLE VIII

INFLUENCE OF GERMINATION ON THE SUGAR CONTENT AND DIASTATIC ACTIVITY OF WHOLE WHEAT, BRUSH, AND GERM ENDS

Moisture when cut	Where dried	Sugar content [1]				Diastatic activity [2]			
		Whole wheat check	Germinated			Whole wheat check	Germinated		
			Whole wheat	Germ end	Brush end		Whole wheat	Germ end	Brush end
%		*Mg.*	*Mg.*	*Mg.*	*Mg.*	*Mg.*	*Mg.*	*Mg.*	*Mg.*
43.6	Sun	76	225	365	75	432	970	1400	610
35.5	"	50	185	225	48	370	970	1295	560
29.0	"	54	184	230	50	340	1075	1370	560
13.0	"	65	140	205	50	245	980	1280	550
13.0	"	55	135	245	55	285	960	1370	600
29.0	Shade [3]	50	130	155	52	488	870	1130	525

[1] Mg. of maltose from 10 g. wheat meal before digestion.
[2] Mg. of maltose from 10 g. wheat meal after digestion at 30° C. for 1 hour.
[3] Wetted four times, 30 minutes each.

The sugar content was increased several times by the process of germination in the whole wheat and the germ ends but not in the brush ends. The increase was greater in the germ end than in the wheat. The brush ends had no more sugar after germination than the whole wheat before germination.

The diastatic activity was increased in all three, most in the germ end and least in the brush end. This means that the enzymes stored in the endosperm are activated by the process of germination. However, since more than twice as much diastatic activity was produced in the germ end as compared with the brush end, the larger amount of the increase in diastatic activity due to germination is due to the enzymes of secretion which are derived from the epithelial layer of cells between the germ and the endosperm.

Summary

Moisture and temperature affect the quality of wheat during harvest because they influence the rate of biological activity in wheat. The sugar content and the diastatic activity in wheat are not influenced unless the amount of moisture is sufficient to start the process of germination. The duration of the wet condition as influenced by conditions of drying is more important than the amount of wetting.

Wheat cut at 29% moisture was not lowered in test weight, but at higher moisture content there was a slight lowering. The percentage of yellow berry was apparently more influenced by late maturity than condition of drying. Drying the wheat cut at high moisture contents slowly, and at a low temperature, produced an increase in diastatic activity, but not in sugar content.

The diastatic activity of the germ and brush ends was not much different and nearly like that of wheat. However, when the germ and brush ends were germinated for 72 hours there were pronounced differences. Both the germ ends and the whole wheat increased in sugar content, but no increase occurred in the brush ends. The diastatic activity was increased in all three, but least in the brush end and most in the germ end.

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A COLLABORATIVE STUDY ON THE USE OF THE WHEAT MEAL "TIME" TEST WITH HARD AND SOFT WHEATS

E. G. BAYFIELD [1, 2]

Ohio Agricultural Experiment Station, Wooster, Ohio

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Correspondence with a considerable number of wheat workers in various parts of the United States and Canada indicated that the whole wheat meal fermentation time test was, or had been, used as an aid for evaluating wheat. Most of the workers contacted were not entirely satisfied with their results. Several stated they believed standardization of the test was needed. A study of the various methods used in their respective laboratories indicated a considerable variation in procedure although all were based upon the technique outlined either by Pelshenke (1930, 1933) or by Cutler and Worzella (1931). Variations in equipment and laboratory facilities accounted for the differences in methods employed by the various correspondents. These various methods made comparison of data between laboratories of doubtful value and indicated a need for standardization. Collaborative testing programs leading toward a uniform procedure have proven both time- and labor-consuming in the case of baking and viscosity tests, and the wheat meal "time" test gave every indication of being of a similar nature.

Because results of several investigators were not particularly favorable to the test, it appeared desirable to the author, before a standardization program was undertaken, that "time" data be compared with recognized tests of longer standing, and their value thus be determined. A review of results obtained by investigators in various parts of the world need not be given here as much of this has been already given by the author elsewhere (1935, 1935a). It must be pointed out, however, that favorable reports have been made by a considerable number of workers, principally plant breeders, in America, Germany, Australia, and New Zealand. A wide range in wheats is therefore included in these reports, and it may be that the test is better

[1] Associate in Agronomy (Cereal Chemist).

[2] For this collaborative study data and samples were received from W. F. Geddes, Dominion Grain Research Laboratory, Winnipeg; A. G. McCalla, University of Alberta, Edmonton; A. G. O. Whiteside, Central Experimental Farm, Ottawa, Ontario; C. O. Swanson, Kansas Agricultural Experiment Station, Manhattan; D. A. Coleman, Bureau of Agricultural Economics, and C. C. Fifield, Bureau of Plant Industry, United States Dept. of Agriculture, Washington, D. C. The opinions expressed and conclusions drawn herein are those of the author.

adapted to some types of wheats than others, due to variations in "gassing power" and protein produced by the wide range in varieties and environments encountered. The present study deals with North American wheats only, and the conclusions drawn will necessarily apply to these wheats alone.

Collection of Samples and Data

Working with soft winter wheats of the same general type as that used by Cutler and Worzella (1931), the author (1935) was unable to reach the same favorable conclusions obtained by these earlier investigators, although an essentially similar procedure was employed. To enlarge the range in wheat strength and to include some material similar to the hard wheats included in the studies reported by Pelshenke (1930, 1933) and by Cutler and Worzella (1931); the author obtained from several sources several series of wheat, either of the hard red spring or of the hard red winter class. These samples had previously been rated for strength (baking test) and protein content by the respective donors who kindly forwarded this information with the samples. The samples included a wide range in protein content as well as varieties of inherently different protein qualities. Such material should provide a good opportunity for studying the strength rating of the samples as given by the whole wheat meal fermentation time test when all samples were subjected to a uniform "time-test" procedure in one laboratory.

Experimental

It has already been mentioned that the various contributors of material in this study used somewhat different procedures in their respective laboratories. Two general types of grinders are in use as well as variations in procedure. Of all the possible variables entering into the test the one most difficult to evaluate is that of the operator who makes the doughballs. Should a standardization program be entered upon, this will be the most difficult variable to place upon a uniform basis.

Collaborative Tests

To obtain some idea as to what may be expected in data originating from various laboratories, a few simple collaborative tests were made. In these tests, samples of both ground and unground wheat were sent to the collaborator with the request that the meal and wheat be tested in the customary way and that a portion of the meal as ground by the collaborator be returned for testing by the author. Thus, two different grindings of each wheat were tested by each laboratory. Results were

very erratic and the data indicated that until the test is further standardized there exists very little use in carrying out collaborative testing of the customary type. It should be pointed out that all the operators entering this interlaboratory testing were experienced in their own particular method.

Bayfield (1935) pointed out the difference in results due to the test vessel walls supporting some doughballs when a 10-g. size ball was used with a 150 cc. beaker, whereas this did not ordinarily occur with soft wheats when a doughball half this size was used. To obtain additional information, samples of wheat and their meals were sent to one collaborator requesting him to time test the meals and his own grindings by two methods:

1. Using 80 cc. of distilled water in 150 cc. low-form beakers, 5.5 cc. of 10% Fleischmann's baker's yeast suspension and 10 g. of meal, and
2. The same method as above excepting that the amounts of yeast suspension and meal were reduced by one-half.

This collaborator used a fermentation temperature of 31–32° C. whereas the author performs the test at 30° C. The collaborator used a Wiley mill equipped with the 1 mm. sieve for grinding. However, his meals were somewhat coarser in appearance than those ground by the author. The time results obtained by this collaborator as well as results (5-g. size) on his meals obtained by the author are given in Table I. The meals were handled as speedily as possible, being mailed in air-tight containers. It is not considered that the time elapsing between grinding of the wheat and testing of the meals was long enough to produce significant differences in the "time" of these samples.

In discussing his results the collaborator concludes that Method 1 (10-g.) "is very unsatisfactory for strong wheats. The ball spreads out over the whole surface and wedges, and hence it seems to be held up for a long time." He also pointed out the greater variations obtained by Method 1 (10-g.) than by Method 2 (5-g.). These points made by the collaborator regarding the tests are essentially the same as the conclusions previously drawn by Bayfield (1935, 1935a) in attempting to evaluate a larger series of wheats.

These interlaboratory tests indicated the futility of attempting to test samples in the usual collaborative manner. Therefore, an alternative method was used whereby wheats of known quality and strength were obtained and all tested by one uniform time-test procedure. Spring wheats from three Canadian sources, hard red winter wheats from Kansas, and soft winter wheats from the collection of the Tri-State Soft Winter Wheat Association in the laboratory of the

TABLE I

TIME DATA OBTAINED BY TWO METHODS IN TWO LABORATORIES

	Method 1 (10-g. meal)			Method 2 (5-g. meal)		
Sample number	1991	1994	1995	1991	1994	1995
Variety	Trumbull	Red Rock	American Banner	Trumbull	Red Rock	American Banner
Meals ground at Wooster						
Time by Collaborator using 31–32° C. temperature						
Replicate 1—min.	49	123	Sample	49	63	Sample
" 2—min.	49	124	lost	48	64	lost
" 3—min.	91	137		52	65	
Time by Bayfield using 30° C. temperature						
Replicate 1—min.	168	215	43	59	102	46
" 2—min.	168	201	44	60	102	46
" 3—min.	167	204	49	75	100	48
" 4—min.	175	200	45	63	96	46
Meals ground by Collaborator						
Time by Collaborator						
Replicate 1—min.	124	58	27	43	56	36
" 2—min.	126	128	28	44	57	36
" 3—min.	132	135	27	44	60	36
Time by Bayfield						
Replicate 1—min.	—	—	—	67	107	40
" 2—min.	—	—	—	70	117	41
" 3—min.	—	—	—	62	109	42
" 4—min.	—	—	—	56	109	40

author were therefore used. Samples were collected in such manner that both variation in protein content as well as variety could be studied.

All samples were ground in a uniform manner on a Wiley mill equipped with a 1 mm. sieve (Bayfield, 1935). Ten grams of meal were used according to Method 1 given in Table I. Where smaller amounts of meal were used the quantity of yeast suspension was reduced proportionately, but the amount of water (80 cc.) and size of vessel (150 cc. beaker) were held constant throughout.

Test with Various Classes, Grades and Varieties of Wheat

CANADIAN HARD RED SPRING WHEATS

The first series of these wheats was received from Dr. W. F. Geddes and consisted of residues from a study reported in a paper by Aitken and Geddes (1934) to which the reader is referred for complete baking and other data. A part of these data together with the obtained time data are presented in Table II.

TABLE II

DATA ON TEN COMPOSITES OF COMMERCIAL CANADIAN HARD RED SPRING WHEAT RECEIVED FROM W. F. GEDDES, WINNIPEG, CANADA

Winnipeg number	Wheat protein[1]	Diastatic activity[1] (flour)	Loaf volume[1,2] (m-p-b)	Loaf volume[1] (basic)	Loaf volume[1] (bromate)	Time[3] 10-g.	Time[4] 4-g.	Time[5] 4-g. tempered
	%	*Mg.*	*Cc.*	*Cc.*	*Cc.*	*Min.*	*Min.*	*Min.*
13269	16.5	104	935	680	810	358	209	240
70	16.1	110	910	650	775	387	221	237
71	15.6	110	855	640	770	356	209	225
72	14.7	110	830	630	770	372	212	226
73	14.4	116	805	595	740	380	214	232
74	13.8	110	795	600	710	356	207	224
75	13.4	114	760	560	675	338	203	229
76	13.0	94	720	570	630	308	199	222
77	12.3	88	670	570	650	284	183	221
78	12.1	100	630	540	600	269	181	220

[1] Data from Aitken and Geddes (Cereal Chem. 11: 487–504).
[2] Loaf volumes from malt-phosphate-bromate formula with 3-hour fermentation period.
[3] Time data according to Cutler-Worzella method (10-g.) excepting temperature of 30° C. used.
[4] Data when beaker gives no support to doughball. Same procedure as [3] save a 4-g. doughball used instead of 10-g.
[5] Meal air-tempered in fermentation cabinet for 12 hours before using.

The baking data given in Table II obtained by the bromate and malt-phosphate-bromate formulae show a good relationship with protein content. The range in loaf volume by the latter formula is larger than the range obtained by the fermentation time test. The tendency for "time" to decrease with decreasing protein content is noticeable but the results are not as consistent as with the baking test. The same trends may be observed in either 10- or 4-g. doughballs. Tempering of the meal produced no particular improvement in results. It was found easier to duplicate results with the smaller sized doughs than with the 10-g. doughballs which, in this series, all received support from the walls of the test vessel. Due to the trouble in obtaining satisfactory checks with the 10-g. doughs this size of doughball was not used further in testing these strong wheats.

The second series of hard spring wheats consisting of one variety, Marquis, was received from A. G. McCalla of the University of Alberta. These samples were all grown under similar environmental conditions in 1933. Table III gives the baking and analytical data received with the samples as well as time figures obtained by the author. It will be observed that both 5- and 4-g. doughballs were run. It was found that some of the 5-g. doughballs gave evidence of receiving support from the test vessel. For such strong wheats apparently 4 g. of meal is as large as should be used with the 150 cc. beaker. Loaf volumes are given for both the basic and bromate formulae. Dr. McCalla in remarking on the two baking formulas used states, "The baking results obtained by use of the basic formula are not considered

TABLE III

DATA ON MARQUIS WHEAT SAMPLES FROM UNIVERSITY OF ALBERTA. 1933 CROP

U. of A. laboratory number	Grade [1]	Wheat protein [1]	Absorption [1]	Basic formula Loaf volume [1]	Bromate formula Loaf volume [1]	Time 5-g.	Time 4-g.
		%	%	*Cc.*	*Cc.*	*Min.*	*Min.*
E 43	1	18.0	68	588	764	209	206
E 17	1	15.1	68	568	685	282	265
E 47	1 Hd.	14.3	70	574	650	248	252
E 36	1 Hd.	13.4	69	556	586	323	298
E 38	1	12.8	69	555	572	276	250
E 45	1 Hd.	11.8	69	509	513	258	242
E 13	1	10.9	69	498	478	243	222

[1] Data from A. G. McCalla, University of Alberta.

TABLE IV

DATA ON A NUMBER OF CANADIAN SPRING WHEAT VARIETIES GROWN AT VARIOUS LOCATIONS IN 1934. SAMPLES RECEIVED FROM CENTRAL EXPERIMENTAL FARM, OTTAWA

Ottawa number	Variety	Canadian origin	Grade [1]	Weight per bushel [1]	Wheat protein [1]	Loaf volume [1, 2]	Crumb texture [1, 3] 1–10	Time 4-g.
				Lbs.	%	*Cc.*	*Score*	*Min.*
34.167	Reward	Winnipeg	2	64.2	13.7	725	8	220
266	Marquis	Indian Head	1 Hd.	64.3	14.5	942	6	183
416	Marquis	Fallis	4	62.9	9.9	518	3 c	60
391	Marquis	Swan River	3	65.7	8.7	492	6 c	206
243	Ceres	Morden	1	63.4	15.6	870	6	188
343	Ceres	Saskatoon	1	62.1	15.5	938	6	184
268	Ceres	Indian Head	1 Hd.	64.6	14.7	875	7	160
168	Ceres	Winnipeg	2	60.4	12.7	662	8	173
193	Ceres	Solsgirth	1	64.8	12.6	707	7	222
418	Ceres	Fallis	4	63.0	10.2	517	2 c	49
393	Ceres	Swan River	3	65.5	9.3	515	6 c	128
244	Huron	Morden	1	62.2	16.6	885	7	175
344	Huron	Saskatoon	2	61.2	16.3	840	8	162
269	Huron	Indian Head	1	63.5	15.1	923	6	130
369	Huron	Lacombe	2	63.9	13.8	673	8	79
194	Huron	Solsgirth	1	64.0	12.8	708	8	187
394	Huron	Swan River	2	65.4	9.5	543	6 c	84
419	Huron	Fallis	4	52.9	8.8	455	0 c	49
245	Garnet	Morden	2	62.1	15.1	758	9	231
270	Garnet	Indian Head	2	63.5	13.9	725	8	188
395	Garnet	Swan River	2	66.8	10.7	455	6 c	240
420	Garnet	Fallis	4	64.5	9.0	450	3 c	91

[1] Data from A. G. O. Whiteside, Cereal Division, Central Experimental Farm, Ottawa, Canada

[2] Baking test formula—Flour—100 g., 13.5% moisture basis
Yeast—3 g. Fleischmann's baker's yeast
Sugar—2.5 g.
Salt—1.0 g.
Malt—0.3 g. (250° Lintner diastatic malt extract)
$NH_4H_2PO_4$—0.1 g.
$KBrO_3$—0.001 g.

[3] c = coarse.

of much value because this formula does not allow the high protein samples to attain anything like optimum development. The loaf volumes by the bromate formula show a high correlation with protein. These samples were taken from a series of 48 in which this correlation was $0.942 \pm .011$."

Examination of the time data shows but little satisfactory relationship exists between time and the remainder of the analytical data presented for these samples consisting of a single variety.

The remainder of the Canadian wheat samples was received from A. G. O. Whiteside, Central Experimental Farm, Ottawa, and consisted of several varieties of differing inherent gluten qualities. In most cases a range in protein content is represented in the samples of these varieties given in Table IV. The strength rating as given by time does not agree well with that given by the baking test (malt-phosphate-bromate formula). Thus, for example, Marquis with 8.7% wheat protein gives a time of 206 minutes and a loaf volume of 492 cc.; whereas another Marquis, with 14.5% protein and 942 cc. loaf volume, gives a time of only 183 minutes. Comparing Garnet with Marquis it will be observed in Table IV that the former variety according to "time" is stronger on the average than the latter although Marquis is recognized as having the superior quality.

Hard Red Winter Wheat Samples

In response to a request, Dr. C. O. Swanson forwarded the samples of Kansas grown wheats, data for which are presented in Table V.

TABLE V

Data for Kansas Grown Hard and Soft Winter Wheats. Samples Received from Agricultural Experiment Station, Manhattan, Kansas

Kansas number	Variety	Section grown	Wheat protein [1]	Flour protein [1]	Absorption [1, 2]	Loaf volume [1, 3]	Color [1]	Time 5-g.	Time 4-g.
			%	%	%	*Cc.*		*Min.*	*Min.*
19512	Kanred	Northwestern Kansas	15.25	13.85	65	1640	98	81	87
19523	"	Eastern "	12.40	10.65	63	1640	100	66	70
19513	Tenmarq	Northwestern Kansas	15.05	13.85	66	1800	100	193	175
19524	"	Eastern "	11.80	10.70	65	1750	99	145	151
19515	Quivira	Northwestern Kansas	15.00	13.65	70	1700	98	84	83
19525	"	Eastern "	12.70	10.75	68	1675	98	59	57
19520	Fulcaster	Morris and Dickinson Counties, Kansas	12.75	11.50	62	1675	100	47	51
19527	"	Coffey and Franklin Counties, Kansas	11.10	9.55	60	1605	99	45	43

[1] Data from C. O. Swanson, Dept. of Milling, Kansas Agricultural Experiment Station, Manhattan.

[2] Absorption determined on super centrifuge.

[3] Baking formula—Flour 250 g.
Yeast 4.4 g.
Sugar 15.0 g.
Salt 4.5 g.
Lard 2.5 g.
Water according to absorption.

These samples and their data are quite interesting in that the time test data agree much better with strength as represented by protein content than was the case with the hard spring wheat samples. The baking test was quite different from that employed by the Canadian workers and this very probably accounts for the small differences obtained in loaf volume. In these samples the time test correctly separates the soft red winter variety, Fulcaster, from the hard red winter varieties such as Kanred. The loaf volumes do not represent the strength anticipated from a knowledge of the protein contents and protein qualities of these different variety samples.

Soft Winter Wheat Samples

The Bureau of Agricultural Economics of the U. S. Department of Agriculture has been interested in the time test for some time. Dr. D. A. Coleman of this Bureau was kind enough to mill and bake several wheat samples, the resulting data are given in Table VI.

TABLE VI

Data for 3 Varieties of Ohio Grown Soft Winter Wheats

U.S.D.A.[1] number	O.A.E.S. number	Wheat[1] protein	Flour[1] protein	Loaf volume[1, 2] Method 1	Loaf volume[1, 2] Method 2	Loaf volume[1, 2] Method 3	Loaf volume[1, 2] Method 4	Loaf volume[3, 4] (regular)	Loaf volume[3, 4] (bromate)	Time[4] 10-g.	Time[4] 5-g.
		%	%	*Cc.*	*Cc.*	*Cc.*	*Cc.*	*Cc.*	*Cc.*	*Min.*	*Min.*
				Variety—Red Rock							
25734	1904	13.80	12.29	498	551	507	490	615	—	**203**[5]	101
25737	2044	10.41	9.12	449	439	461	421	565	532	**176**[5]	54
25740	2054	9.70	8.30	452	452	441	433	595	550	**154**[5]	47
				Variety—Trumbull							
25733	1991	13.64	12.70	464	510	490	484	665	—	**171**[5]	61
25736	2041	11.24	10.01	449	473	455	430	585	557	53	46
25739	2051	10.94	9.90	443	471	467	449	527	550	41	34
				Variety—American Banner							
25735	1995	12.60	11.22	447	461	464	441	610	—	49	46
25738	2045	10.67	9.42	453	462	441	452	550	590	34	40
25741	2055	9.42	8.80	470	464	481	439	555	485	29	34

[1] Data from D. A. Coleman, Bureau of Agr. Economics, U. S. Department of Agriculture, Washington, D. C.
[2] Method 1—A. A. C. C. basic procedure with 54% absorption, Hobart-Swanson mixer.
2—As Method 1 plus 0.002 g. potassium bromate.
3—As Method 1 but using 3.5% sugar and 0.5% malt.
4—As Method 1 but using 5.5 minutes mixing.
[3] For formula see footnote 1, Table VII. Flour from same wheat but not milled by U. S. D. A. A KitchenAide mixer equipped with paddle used for mixing.
[4] Data by author in J. Am. Soc. Agron. **27**: 241-250.
[5] Bold-face type refer to samples receiving support from wall of test vessel.

These samples were a part of those included in a previous paper by the author (Bayfield, 1935) and represented a considerable range in protein content in three varieties of differing gluten characteristics.

Of the various baking procedures employed on the samples by Dr. Coleman, his grain and texture scores favored Method 2 (basic plus bromate) much more than by the other methods. Many of the actual differences obtained in loaf volumes are too small to be significant. Unfortunately the bromated baking series according to the procedure of the Tri-State Soft Wheat Improvement Association is not complete. No explanation is apparent for the unexpected baking strength in the lowest protein Red Rock and American Banner samples when baked in either laboratory. The data are indicative of the need for a standard baking test suitable for soft wheat flours.

The time data show two things: first, the disturbing influence of the test vessel when the 10-g. size of doughball is used; and second, that protein content influences time. In using the time test, therefore, it will be advisable to consider the total *quantity* of protein if an estimate of gluten quality is wanted. Pelshenke (1930, 1933) recognizes this factor and his index of gluten quality is obtained by dividing "time" by the amount of gluten present.

According to Clark and Quisenberry (1933), Trumbull wheat was the third most important United States soft red winter variety in 1929. Considerably over half of the total Ohio acreage was sown to this variety. It was, therefore, selected for studying the effect of varying protein content upon time in this class of wheat. Twenty samples from each of the 1933 and 1934 crop seasons were selected, the wheat originating in widely distributed sections of the Tri-State territory. Table VII gives the resulting data. Two sizes of doughballs were used in testing the 1933 samples. With the 10-g. size it will be observed that Nos. 1991 and 2371 received support from the test vessel and their times were prolonged about 100 minutes. The 5-g. doughballs very seldom have their times interferred with in this way.

The 1934 soft red winter wheat crop of most of Ohio, Indiana, and Michigan was exceptionally high in protein content. Many mills had trouble obtaining their requirements of low protein wheat for cake flour production and similar purposes. The data in Table VII indicate that protein is producing but little influence upon time; and if a miller were to purchase and bin his wheat on a "time" basis, he undoubtedly would experience trouble with his bakery customers. For example, Sample 2595, with a protein content of 15.9%, gave a time of 38 minutes. Such wheat in practise has been found less desirable for soft wheat flour production than samples, such as No. 2522, with a protein content of 10.3%; although the time in this case is somewhat higher (42 minutes).

To study the effect of variety upon time, samples from the 1932 and 1933 crops were used. At each of 10 Ohio locations in 1932 and at

TABLE VII

DATA FOR TRUMBULL SOFT RED WINTER WHEAT GROWN IN TWO SEASONS AT VARIOUS OHIO, INDIANA, AND MICHIGAN LOCATIONS

1933 Crop Samples					1934 Crop Samples				
Sample number	Wheat protein	Loaf volume [1] (regular)	Time [3] 10-g.	Time 5-g.	Sample number	Wheat protein	Loaf volume [1] (regular)	Loaf volume [2] (bromate)	Time 5-g.
	%	*Cc.*	*Min.*	*Min.*		%	*Cc.*	*Cc.*	*Min.*
1991	14.0	665	**168**	61	2595	15.9	540	565	38
1971	12.4	630	54	56	2442	15.0	573	545	44
2331	11.6	633	37	41	2424	14.6	565	563	50
2321	11.6	590	37	46	2558	14.1	635	610	48
1981	11.4	580	53	60	2381	14.0	575	560	78
2341	11.3	662	40	46	2579	13.8	545	620	38
2041	11.2	585	48	46	2396	13.0	520	525	46
2011	11.1	647	47	50	2616	12.8	513	490	42
2371	11.1	600	**153**	55	2609	12.7	550	580	42
1961	11.0	650	63	62	2566	12.1	575	535	47
2031	11.0	605	47	50	2406	12.0	530	528	47
2251	10.9	557	44	50	2542	11.9	590	533	52
2051	10.9	527	35	34	2623	11.8	520	530	40
2071	10.8	575	35	40	2386	11.4	533	520	50
2361	10.6	645	46	50	2602	11.2	530	520	37
2061	10.6	630	39	45	2588	11.0	560	530	31
2021	10.5	610	43	49	2391	10.9	560	553	39
1951	10.4	655	59	67	2550	10.8	555	538	47
2351	9.6	550	46	52	2401	10.8	568	508	46
1941	9.0	540	49	57	2522	10.3	510	438	42

[1] "Regular" baking test formula: Flour—100 g. on 15% moisture basis
Sugar—5.0 g.
Yeast—3.0 g. (Fleischmann bakers)
Salt—1.0 g.
Water—(distilled) to correct absorption.

[2] As in "regular" baking test formula with 0.001 g. potassium bromate added.

[3] Bold faced figures refer to samples with exaggerated "time" due to test vessel supporting doughball.

17 Ohio and Michigan locations in 1933, a uniform series of 10 varieties was grown. Each of these 270 samples was time tested using 5-g. doughballs in quadruplicate. The results for the two years are given in Table VIII. It may be observed that the time test will readily detect the weak white variety, American Banner, from the strong soft red variety, Red Rock, in either year. Season apparently affects some varieties more than others. Thus, Bald Rock was relatively stronger in 1932 than in 1933. The relative strengths of the different varieties according to time were different from those given by the baking test. Thus, Nabob and Michigan Amber over several seasons of baking have proven weaker than Trumbull (Bayfield, 1934a), yet according to time

TABLE VIII

TIME DATA FOR 10 VARIETIES GROWN IN 1932 AND 1933

Variety	Average for 10 Ohio 1932 locations	Average for 10 Ohio 1933 locations	Average for 17 1933 locations
	Min.	*Min.*	*Min.*
Trumbull	52.3	53.1	51.8
Nabob	56.4	56.2	56.5
Fulhio	50.0	54.0	52.1
Red Rock	68.7	65.0	67.2
American Banner	34.2	36.1	34.5
Bald Rock	65.6	55.4	56.1
Michigan Amber	62.3	60.4	62.8
Kharkov	50.6	47.5	45.5
Fultz	56.4	61.3	58.9
Gladden	40.8	53.3	49.1
Av. all varieties	53.7	54.2	53.5

they are stronger. No reason for this discrepancy is known, but it may possibly prove to be due to differences in the diastatic powers of these two varieties.

A limited amount of statistical analysis was made on the 100 samples tested from the 1932 crop. Additional data of this type for these samples have been presented in a previous paper (Bayfield, 1934). The correlation coefficients given in Table IX indicate that time

TABLE IX

SOME CORRELATION COEFFICIENTS OBTAINED ON THE 1932 SAMPLES

Factor correlated with	Correlation coefficient, r_{xy}, with	
	Percentage wheat protein	Time—5-g.
Loaf volume (bromate)	+.8289	+.4227
Viscosity, 9 cc. acid	+.6857	+.4436
Percent hard kernels[1]	+.7482	+.3430
Percent soft kernels[1]	−.7058	−.3633
Fermentation index "A"	−.2977	−.0438
Percent absorption	+.3763	+.6159
Time, 5-g. doughballs	+.3388	—
Flour protein	+.9922	—

[1] Texture analysis made with aid of bottom illumination.

(5-g. doughballs) is correlated less than wheat protein with the several other measurements.

Conclusions and Summary

The data accumulated in this study indicate that the wheat meal fermentation time test is very sensitive to modifications in technique.

This leads to the belief that it will prove very difficult to standardize the test so that comparative results for the same samples may be obtained from different laboratories. Much improvement can, however, be brought about by standardizing grinding and other equipment.

The size of doughball used is of great significance, and the author is of the opinion that a doughball made from 4 g. of meal will prove satisfactory when 150 cc. low-form beakers are used as test vessels. This size of doughball should permit the testing of maximum strength found in North American wheats. It is desirable that the fermenting doughball receive no support from the walls of the test vessel.

The time data presented for both hard red spring and soft winter wheats show that these data do not give as reliable a strength rating for the samples as that given by the baking test. Considering all data, the baking test still remains the best all-round test for strength *provided* a baking procedure which brings out the characteristics of a flour is used.

While the data presented in this paper are not very favorable to the time test, the author is of the opinion that the test will prove helpful to the wheat breeder as a low cost aid in segregating the undesirable extremes in breeding material at an early stage before sufficient grain is available for a milling and baking test. Even during these early stages the time test should be carried out in conjunction with a protein determination. This is particularly true if an estimate of gluten quality is desired, seeing that amount of protein effects time to a significant extent. For differentiating between new wheats in the median group a more refined testing method than the time-test will be needed. For testing of 100-g. lots of wheat the newly developed micro mill of Geddes and Frisell (1935) has given promising results. Considerable information about the gluten characteristics of a sample may be obtained bv observing the behavior of the doughball during the test. Unfortunately these observations do not appear in the "time" recorded.

From the data accumulated to date it appears that mills and purchasers of wheat will find it advisable to observe considerable caution in employing the test. Such buyers of wheat have no shortage of material available for test purposes, and more delicate and refined methods than the wholemeal fermentation time test are available for them to use.

Acknowledgments

The author wishes to express his appreciation to the various donors of wheat and of accompanying analytical and baking data obtained on these samples. The providing of these samples of known characteristics has made this study possible.

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REPORT OF THE 1934–35 PIE FLOUR COMMITTEE

C. B. KRESS, *Chairman*

Sperry Flour Company, San Francisco, California

(Read at the Annual Meeting, June 1935)

The purpose of the collaborative study was to further perfect the method for testing pie flours and to apply, if possible, the general methods of flour testing to various pie flours.

The committee comprised six members. Three were associated with pie manufacturers and three were chemists for milling concerns. The committee started with the procedure of last year and, after correspondence, made minor changes and introduced a standard pie filling which was used in filled pies. Ten flour samples were studied by each member—four from the Pacific Coast, two from Idaho, and four from Ohio and Illinois.

Directions for Collaborative Studies on Pie Flours

Chemical determinations: Ash and protein analyses corrected to a 13.5% moisture basis.

Color: Slick, dip in water, let dry at room temperature for one hour, and report—(*a*) clearness, *i.e.*, specky or clear, and (*b*) shade, *i.e.*, white, yellow, slightly yellow, gray, slightly gray, or dark.

Viscosity determinations: Use 20 g. flour (13.5% moisture basis) and 100 cc. of distilled water at 65° F. Place the flour in a mortar with 40 cc. of water and work the flour into a suspension with the aid of a pestle. Add the remainder of the water to the mortar while mixing and mix until a smooth suspension has been obtained. Transfer the suspension to a MacMichael viscosimeter and read its viscosity at once. Use a No. 30 wire and operate the bowl at 12 r.p.m. Then add 1 cc. of normal lactic acid, stir, and again read the viscosity. Add three successive 2 cc. portions of normal lactic acid to the flour-in-water suspension until a total of 7 cc. of acid has been added, stirring the suspension after each addition of acid, and making viscosity readings after each addition of acid.

Report the viscosity readings as

Acid, *Cc.*	0	1	3	5	7
Viscosity, ° *MacM.*	15	25	30	32	32

Hydrogen-ion concentration: Test the pH of the flour either electrimetrically or colorimetrically.

Baking test: Formula—100 g. flour, 13.5% moisture basis; 3 g.

yeast; 1 g. salt, 2.5 g. sugar. Determine absorption at time of mixing. Mix the dough at 82° F. and ferment at 82° F. Make two doughs of each sample. Classify dough as to soft, elastic, or tough. Use three and four hour fermentation periods, dividing the dough times as follows:

	3-hour dough	4-hour dough
First punch	105 min.	140 min.
Second punch	50 min.	65 min.
Third punch	25 min.	35 min.
In pan	Until ready—about 60 min.	Until ready—about 60 min.

Bake at 450° F. for 25 minutes. Measure volume of loaf in cubic centimeters. Score bread as follows: Crumb color, texture of crumb, grain of crumb, crust color, oven spring, and baking strength.

Pie Baking Procedure

Formula for bottom shells and top crusts:

Flour	2.5 lbs.
Shortening	1.5 lbs. (Crisco melting point 104° F.)
Salt	1.25 oz.
Water	1 pt. (at 45° F.)

Mixing procedure: Mix the shortening and flour together with a fork or heavy beater (such as small beater of Hobart Mixer) until the shortening is in pieces of about 0.5 in. in diameter. This will require about 5 minutes. Dissolve the salt in water and add to the flour and the shortening. Mix lightly with a fork or beater until a partially mixed mass is reached—2 minutes is sufficient time for this. Put the dough away as soon as mixed in a refrigerator at approximately 50° F. for 24 hours.

Top crust: Take out sufficient dough to make the top crusts (10 oz. for each). Roll to 1 in. thickness, fold over twice in opposite directions, producing three layers, put away in refrigerator for 24 hours.

Bottom shells: After standing 24 hours in refrigerator, take out and make shells as follows:

Take out 11 oz. of the pie dough, press into a ball and roll out on a floured canvas to 1/8 in. thickness. If rolled to approximately 12 in. diameter it will be 1/8 in. thick. Now fold twice so that there will be four sheets each 1/8 in. thick. Make this 5 inches square by folding the edges. Roll this piece out to a thickness of 1/8 in. again (12 in. diameter), fold as before and roll out a third time to 1/8 in. thickness (11–12 in. diameter). Lay this sheet of dough over an inverted pie pan. Press down firmly all over the surface of the pan. Trim off the dough around the edge with a knife (trimmings should weigh approximately 5.5 oz.). With a fork prick many small holes

through the sheet of pie dough covering the bottom of the pie pan (to let out the steam), press firmly to the pan at the edges, and let stand 30 minutes to dry. Then cover with another pie pan pressed down firmly, and bake at 425° F. for 20 to 25 minutes. Then, ten minutes after putting it in the oven, remove the pie tin from the top so that the bottom will brown. Examine the pie shells the next day. At least two shells should be made from each sample of flour.

Procedure for Making Fruit Filling

Make the following artificial fruit filling. The reason for this is to have a mixture that will have the acidity of a fruit pie filling and also its liquid condition, and a mixture that will be uniform and not subject to the variation in acidity that would be experienced if actual fruit was used. Use the following ingredients:

Water	2 qts.
Sugar, granulated	3 lbs.
Corn-starch	8 oz.
Citric acid crystals	½ oz.
A trace of water-soluble color, if desired	

To prepare the filler mix 12 oz. of the cold water with the corn-starch to a smooth paste. Heat the balance of water to boiling together with citric acid and half of the sugar. Take off heat and immediately stir in the starch suspension. Continue stirring until maximum viscosity is reached. Add the balance of the sugar, stirring until the mass is smooth. Cool in a water bath with constant stirring (to prevent lumps forming) to 70° F. The filling is now ready to use.

Roll out a piece of pie dough as for the empty shells. Place in a pie pan, press down well and trim. Add filling to about 1/4 in. of top of pan.

For making the top crust take a piece of the dough as described above, roll out only once to 1/8 in. thickness, wet the edge of bottom crust with water and cover with sheet of top crust, press down edge tightly all around and trim. Cut a hole in center to let out the steam. Bake at 425° F. for about 30 minutes or until crust is sufficiently brown.

In scoring the pie crust the following qualities are to be desired:

Baked through—is it well baked through, or soft and doughy?
No shrinkage from edge of pan—score none, little, or much.
Golden brown color—score light, slightly brown, golden brown.
Flaky—score flaky, compact, or granular.
Tender—score tender or tough, more or less.
Crisp—score crispness or soft.
Will not turn soggy within a reasonable time—score after 12 or 16 hours and note how far crust is penetrated by filling.

TABLE I

Results of Chemical and Baking Tests

Sample number	Collaborator number	Protein	Ash	Max. viscosity	pH	Flour color	Absp.	Volume		Crust color		Crumb color		Texture		Break and shred	
								3 hr.	4 hr.	3 hr.	4 hr.	3 hr.	4 hr.	3 hr.	4 hr.	3 hr.	4 hr.
		%	%	°*MacM.*			%	*Cc.*	*Cc.*	*Score*	*Score*	*Score*	*Score*	*Score*	*Score*		
1[a]	1	6.2	0.45	22	6.20	Y.	58	483	455	Lt. br.	tan	Y.	Y.	coarse	V. coarse	P.	V.P.
	2	6.6	0.45	17		Y.	57	440	475	pale	pale	Y.	Y.	smooth	coarse	P.	V.P.
	3	6.9	0.44	18	6.00	99 g.y.		424	416	pale	pale	100 Y.	99 Y.	coarse	coarse	P.	P.
	4																
	5	6.7	0.45		5.70	Cr.											
2[a]	1	7.4	0.48	28	5.73	Cl. w.	58	513	478	Lt. tan	Lt. tan	Dull wh.	Dull wh.	harsh	tight	P.	P.
	2	7.4	0.48	20		Cl. Sl. Y.	58	400	460	pale	pale	97 wh.	97 wh.	99	99	P.	P.
	3	7.4	0.47	25	5.50	100½ C. Y.		367	357	pale	pale	99 g. w.	98 g. w.	coarse	coarse	P.	P.
	4																
	5	7.3	0.48		5.30	Lt. cr.	59										
3[b]	1	8.2	0.34	37	5.10	V. W.	59	453	388	pale	pale	Wh.	D. wh.	harsh	tight	P.	V.P.
	2																
	3	8.5	0.34	48	5.00	101 g. w.		310	293	V. pale	V. pale	100 g. w.	100 g. w.	firm	firm	P.	P.
	4																
	5	8.1	0.32		5.10	V. lt. cr.											
4[b]	1	9.5	0.38	62	5.23	Cr.	59	463	360	pale	V. pale	D. wh.	D. wh.	tight	V. tight	P.	V.V.P.
	2							342	326	V. pale	V. pale	100 g. w.	99 g. w.	firm	firm	P.	P.
	3	9.8	0.36	82	5.10	100 c. w.											
	4																
	5	9.5	0.35		5.10	Lt. cr.											
5[c]	1	9.1	0.42	50	6.01	Y.	62	498	445	Lt. br.	tan	D. Y.	D. Y.	harsh	Harsh, tight	P.	V.P.
	2																
	3	9.0	0.41	59	6.00	99 g. w.		407	375	pale	pale	98 Y.	97 Y.	coarse	coarse	P.	P.
	4																
	5	8.8	0.40		5.80	Cr.											

[a] Pacific Northwest soft white flour.
[b] Ohio wheat flour.
[c] Minneapolis flour.

TABLE I—*Continued*

Sample number	Collaborator number	Protein	Ash	Max. viscosity	pH	Flour color	Absp.	Volume		Crust color		Crumb color		Texture		Break and shred	
								3 hr.	4 hr.	3 hr.	4 hr.	3 hr.	4 hr.	3 hr.	4 hr.	3 hr.	4 hr.
		%	%	*MacM.*			%	*Cc.*	*Cc.*	*Score*	*Score*	*Score*	*Score*	*Score*	*Score*		
6[e]	1	9.2	0.45	48	6.10	V. y.	59	585	498	Br.	tan	V. y.	V. y.	V. harsh	Harsh, tight	F.	P.
	2																
	3	9.2	0.44	52	6.00	100 Y.		424	420	pale	pale	99 Y	99 Y.	coarse	coarse	P.	P.
	4																
	5	9.0	0.41		5.70	Y.											
7[a]	1	6.9	0.43	25	6.20	Cr.	58	523	495	Lt. br.	pale	Y.	Y.	V. harsh	Harsh, tight	F.	P.
	2	7.5	0.44	24		Sl. Y.	55	505	495	pale	pale	100 Y.	100 Y.	99	99	P.	P.
	3	7.0	0.40	26	6.00	99 g. y.		424	407	pale	pale	100 Y.	99 Y.	coarse	coarse	ragged	ragged
	4																
	5	6.7	0.41		5.70	Cr.											
8[a]	1	7.9	0.41	35	6.20	Sl. Y.	58	548	473	Lt. br.	tan	Y.	Y.	harsh	tight	F.	P.
	2	8.1	0.41	30	6.00	Cl. Sl. Y.	54	475	460	Lt. br.	Lt. br.	102 Cr.	102 Cr.	99	99	P.	P.
	3	8.0	0.40	42		100 Y.		424	416	pale	pale	100 Y.	100 Y.	coarse	coarse	P.	P.
	4																
	5	6.2	0.41		5.70	Cr.											
9[d]	1	8.9	0.78	17	6.30	D. V. Y.	57	360	373	Br.	Lt. br.	D. Y.	V. D. Y.	tight	tight	P.P.P.	P.P.P.
	2	9.0	0.40	10		dark	54	375	400	D. br.	D. br.	90 D.	90 D.	92	92	P.	P.
	3	9.0	0.77	13	6.00	93 D. Y.		362	357	pale	pale	90 Y.	90 Y.	V. coarse	V. coarse	P.	P.
	4																
	5	8.7	0.78		6.10	Y.											
10[d]	1	8.1	0.46	26	5.70	Wh.	58	458	438	pale	pale	Wh.	D. wh.	heavy	tight	P.	P.
	2	8.0	0.46	27		Cl. wh.	53	400	430	Lt. br.	Lt. br.	98 Wh.	98 Wh.	100	99	F.	P.
	3	8.2	0.46	31	5.50	100 g. w.		300	290	pale	pale	98 g. w.	97 g. w.	firm	firm	P.	P.
	4																
	5	75	0.46		5.50	V. lt. cr.											

[d] Idaho soft white flour.

TABLE II—Results of Pie Crust Tests

Sample 1	Pie Crust tests—shells						Citric acid-sugar-corn starch (filled pies)			
	Baked Through	Shrinkage	Color	Flakiness	Tenderness	Crispness	Top crust soaked	Bottom crust soaked	Shrinkage	Gen. condition
Collaborator 1	good	none	G. brown	Very and puffed	Very tender	crisp	⅓	½		fair
2	fair	none	Brown	flaky	V. tender	crisp	Crust not soggy 12–16 hrs.			
3	good	little	Lt. g. brown	flaky	tender	crisp	Crust slightly soggy			
5	good	none	G. brown	flaky	tender	crisp		½		
Sample 2										
Collaborator 1	V. good	none	Brown	Very—compact	Very tender	V. crisp	⅓	½		good
2	fair	none	Sl. brown	flaky	tender	crisp	Crust not soggy 12–16 hrs.			
3	Sl. soft	slight	Uneven—pale center	flaky	tender	Sl. soft	Quite soft and soggy			
5	good	none	Sl. brown	flaky	tender	crisp		½		
Sample 3										
Collaborator 1	Very good	slight	Sl. brown	flaky	V. tender	V. crisp	little	¾		
2										
3	good	considerable	pale	V. compact	Sl. tough	Sl. soft	Soft and soggy			
5	good	none	light	flaky	tender	crisp		⅓		
Sample 4										
Collaborator 1	V. good	much	Gld. brown	V. flaky	excellent	excellent	½	½		Crust tough
2										
3	Soft center	considerable	pale	Sl. compact	Sl. tough	2 Sl. soft	Soft and soggy			
5	good	none	G. brown	flaky	tender	crisp		⅓		
Sample 5										
Collaborator 1	V. good	none	Gld. brown	V. flaky	V. good	V. good	⅓	½		
2										
3	good	little	Even g. brown	flaky	tender	crisp	Good condition			
5	good	little	light	flaky	Sl. tough	crisp		⅓		
Sample 6										
Collaborator 1	V. good	none	Gld. brown	excellent	V. good	V. good	⅓	½		
2										
3	good	little	Even g. brown	flaky	Sl. tough	crisp	Good condition			
5	good	none	G. brown	flaky	tender	crisp		⅓		
Sample 7										
Collaborator 1	V. good	none	Gold brown	very	excellent	excellent	⅓	⅔	none	Still tender
2	fair	none	Sl. brown	flaky	V. tender	crisp	none	none		
3	good	little	Lt. g. brown	flaky	V. tender	V. crisp	Good condition			
5	good	none	G. brown	flaky	tender	crisp		½		
Sample 8										
Collaborator 1	V. good	none	Gold brown	very	very	very	⅓	¾		Still tender
2	fair	none	Sl. brown	flaky	tender	crisp	none	none		
3	good	little	G. brown	flaky	tender	crisp	Good condition			
5	good	none	Sl. brown	flaky	Sl. tough	crisp		½		
Sample 9										
Collaborator 1	V. good	none	Gold. brown	very	very	fair	⅓	¾	none	Still tender
2	V. good	none	brown	flaky	tender	soft	none	none		
3	Little soft	little	Sl. dark g. brown	flaky	tender	crisp	Soft and soggy			
5	good	none	G. brown	flaky	tender	crisp		⅓		
Sample 10										
Collaborator 1	V. good	little	Gold brown	very	very	good	⅓	¾	none	Still tender
2	Poor—doughy	none	Lt. brown	flaky	tender	crisp	none	none		
3	good	slight	Lt. brown	compact	Sl. tough	Sl. soft		soggy		
5	good	none	light	flaky	tender	crisp		½		

TABLE III

PIE CRUST QUALITY—GENERAL CONCLUSIONS AS COMPILED FROM ALL COLLABORATIVE RESULTS

Sample number	Baked through	Shrinkage	Color	Flaking	Tenderness	Crispness	Soaking and toughening of crust	Bleaching	General quality
1	Good	None	G. br.	Good	V. tender	Crisp	Fair	Unbleached	Good
2	Fair	None	Lt. br.	Good	V. tender	Crisp	Good	Bleached	Good
3	Good	Little	Light	Good	Tender	Crisp	Fair plus	Bleached	Good
4	Good	Too much	G. br.	Good	Tender	Crisp	Fair	Bleached	Fair
5	Good	Ltle	G. br.	V. good	Tender	Crisp	Fair	Unbleached	Fair
6	Good	None	G br.	V. good	Tender	Crisp	Fair	Unbleached	Good
7	Good	None	G. br.	V. good	V. tender	V. crisp	Good	Unbleached	V. good
8	Good	None	Lt. br.	V. good	Tender	Crisp	Fair plus	Unbleached	Good
9	Good	None	G. br.	V. good	Tender	Sl. soft	Fair	Unbleached	Fair
10	Good	Little	Lt. br.	V. good	Tender	Crisp	Good minus	Bleached	Good

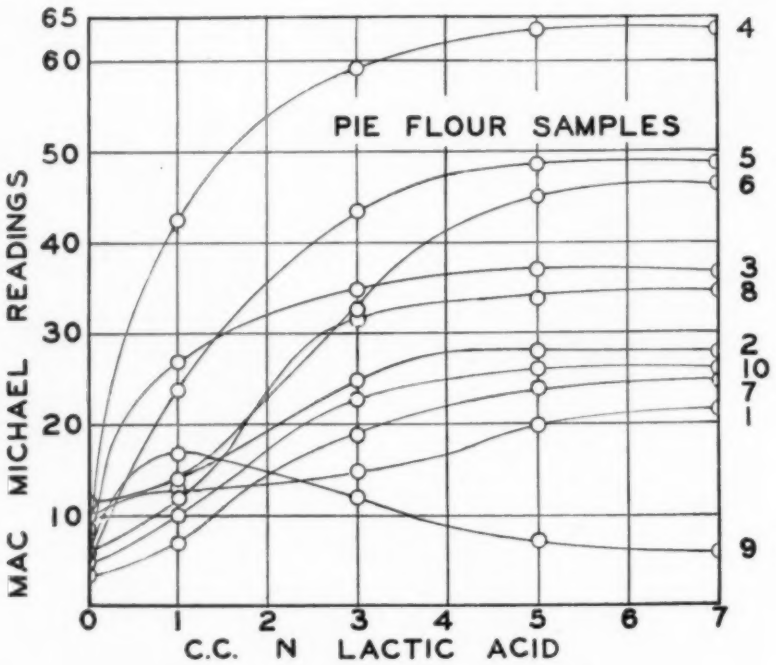

Fig. 1. Viscosity tests—collaborative samples, 1935.

Supplementary Tests Using Peach Filling in Place of Artificial Filling

Canned peaches have a low and uniform pH value and make a pie that can be eaten as well as give a more commercial test.

Directions: Use 1 qt. can standard grade cling peaches. Drain off syrup (should be 8 oz.). Add 2 oz. sugar to 8 oz. syrup, bring to a boil, take from heat and add 1/2 oz. corn-starch mixed with 2 oz. cold water. Stir thoroughly and add to this the peach fruit previously cut into small pieces. Let cool and put into pies.

The results obtained by the collaborators with respect to chemical tests, viscosity determinations, bread baking tests, and pie crust quality tests are shown in Tables I, II, and III, and in Figure 1.

General Conclusions

The committee is satisfied with the pie-dough formula and procedure for making pie shells. No single formula will meet all conditions as some technicians use more shortening and others less. It is felt that the method described is severe enough to eliminate a wholly unsuitable pie flour. It probably will be necessary to reduce the shortening 10 to 15% if it is desired to magnify small differences within the acceptable range.

The compounded filling made from citric acid, corn-starch, and sugar is scientifically correct and does accurately show differences in soaking of pie crust. The sugar content is standard and the pH value is standard, both of which affect the soaking of both top and bottom crusts. It is felt that this method gives good information. However, the *filled pie* is the most important part of the test. It is considered that the filled pie is the best test for *shrinkage, toughness* of top and bottom crusts, and soaking of crust. The *toughness* of the top and bottom crusts of the filled pie is of prime importance and can best be judged in the filled pie.

For all the above reasons it is believed that a filling made more like a commercial pie (such as a peach pie) is better. Such a filling is not subject to standardization as closely as the citric acid filling, but it is believed that the advantages of being able to taste the pie are so important that a peach filling is favored.

The protein, ash, and moisture content of pie flours as well as viscosity tests are certainly important in evaluating a pie flour.

The baking test gives good information but is not absolutely necessary. Flours having soft wheat characteristics are advisable for pie flours. The baking test will properly place a flour in this respect. With the character of the flour located, it is apparently desirable to have a soft wheat flour that will produce a loaf of fair volume. A flour producing a dead appearance and extremely small volume is not desirable. The A.A.C.C. baking test with a 3-hour fermentation time is recommended, and it is not considered necessary to have a time differential.

The *viscosity* test apparently gives the best information of any single test. As shown in the viscosity curves of these samples, the best flours for pie crust purposes start with a medium viscosity, show a gradual rise to a medium low viscosity. Flours which rise rapidly and finish at a fairly high point are not desirable. Similarly,

flours of abnormally low viscosity are not desirable. Samples Nos. 4 and 5 were high viscosity flours and graded Fair; also sample No. 9 had an abnormally low viscosity and graded Fair. The other samples of flour had medium viscosity characteristics and all graded Good. The best flour sample was No. 7 having medium low viscosity characteristics.

pH results and granulation tests were not of apparent correlation with pie flour quality, and it is believed they are not necessary in a pie-flour test.

Recommendations

The committee recommends:

That the pie flour collaborative tests be continued.

That studies be continued on filled pies to arrive at a generally satisfactory standard pie filling that will incorporate all the advantages of the citric acid filling and the peach filling.

That viscosity comparisons be continued, as this looks quite hopeful.

That the baking test be made to determine the general quality of a flour; but that only one fermentation time is necessary. A 3-hour fermentation is recommended.

That the filled pie is the most important, and by all means the test should include a filled pie.

EXPERIMENTAL BAKING PANS[1, 2]

C. F. DAVIS, L. E. LEATHEROCK, and H. W. PUTNAM

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The primary purpose of this report is to re-emphasize the important influence the baking pan and its condition have on the results obtained by the standard A. A. C. C. Baking Test.

In a preliminary attempt to do some collaborative test baking, several Pioneer Section members exchanged baking pans and found that the various pans in use, although of nearly the same dimensions, gave results of such pronounced differences that with other factors constant the pans alone could be made to give loaves of entirely different types and highly significant volume differences. In two laboratories using tall form A. A. C. C. pans of spotless metal, one set of pans consistently gave 10% to 13% more volume than the other set when both sets were used in either laboratory. Two possible explanations for this difference are that one set of pans was improperly broken-in, although all pans had been in use for two years, or that there was a difference in the two sheets of metal from which the pans were cut. Old, darkened tin pans from one laboratory gave loaves of Type J (Blish, 1928) compared with Type F loaves baked in a newer, official, tall form 2XX tin. Tall form aluminum pans used in one laboratory were reported to have given an improvement in general loaf characteristics while the aluminum pans used by another laboratory were reported to have given decreased loaf volume and poorer general loaf characteristics, compared in both cases with the tall form 2XX tin pans already in use. Comparative bakes in one laboratory using the two sets of standard pans proved the aluminum pans to give but intermediate loaf characteristics and one set of the standard tin pans to be sufficiently darkened to give inferior loaves.

Two types of experimental baking pans are described in the official A. A. C. C. Baking Test (Geddes, 1934). A few of the advantages of the low form pan are set forth in the outline. The tall form pan has been in general use since the small loaf procedure was described in detail by Herman and Hart (1927), who found the low form pan to give 15.4% greater loaf volume, a duller crumb, and a more spherical grain structure than the tall form pan.

Treloar and Larmour (1931) using a low form pan found that there

[1] A report by the Pioneer Section Research Committee.
[2] Subcommittee report of the 1934–35 Committee on the Standardization of the Experimental Baking Test.

was a significant increase in loaf volume as compared to the tall form pan, that the grain was more rounded, but the loaf volume variability was not significantly different.

Merritt, Blish, and Sandstedt (1932) studied the relative advantages of the two pans and found that the differential tests imposed on flour by oxidizing agents and fermentation were as easily detected in one pan as in the other. The differentiation of flours with respect to grain and shred was more pronounced in the tall form pan. Their data show slightly less loaf volume variability for the low form pan.

Geddes and Sibbitt (1933) found greater variability between replicates, and variations in crumb texture between the upper and lower portions of the tall form loaves which led them to prefer the low form tins. The volume was significantly larger in the low form to permit a wider range for flour differentiation, also the doughs were easier to mold.

In the history of the A. A. C. C. Baking Test (Brooke and Sherwood, 1928) there are many sizes and shapes of baking pans advocated. Early in the progress of American test baking, A. W. Howard suggested a deep pan to make the test severe and subject the flour to a condition which if overcome would indicate the flour was good or strong. Many laboratories later used deep pans and the tall form A. A. C. C. pan is perhaps a contraction of these earlier deep pans in which larger charges of dough were baked. No doubt the severeness of the test condition imposed by the large tall type pan is magnified when the ratio of dough surface to mass of dough is increased as in the small tall pan. Moen (1935) suggests the idea that the A. A. C. C. baking test is too severe in its differentiations of grain characteristics, especially with a lean formula. It would be interesting to see this work duplicated with low form A. A. C. C. pans to determine to what extent the severe condition was caused by the tall form pan used. The question arises as to whether the baking pan is a logical place and manner to impose a severe condition in an experimental baking test when supplemental formulas and methods are available for detecting flour differences and characteristics.

In order to learn more of the baking pan effect on the A. A. C. C. baking test, official dimension tall and low form pans of different metals and gage were obtained and used in a large number of bakes. The metals included 2XX and 4XXXX tin, black metal (velvediron) 22, 24, and 26 gage, copper, aluminum, and spotless metal. Without giving all the details in regard to the different types of metal as they affected the baking results, it is sufficient to state that the 2XX tin as outlined in the official A. A. C. C. baking procedure gave the most satisfactory results.

A series of flours were baked in the official tall and low form pans in a comparative manner and subjected to the following conditions: Each pan was greased in as nearly the same manner as possible, which caused an average loaf volume decrease of 9.4% for the tall form pan and 3.7% for the low form pan. Each type of pan was darkened (as a breaking-in substitute) in an identical manner which caused a loaf volume decrease of 4.5% in the tall pan, but there was no volume decrease in the low form pan. When light and heavy gage black metal pans were used the heavy gage decreased the loaf volume in the tall form pan 10% to 12% and changed the loaf from Type F to J in appearance, while volume in the low form pan was decreased 3% to 4% and loaf type was not materially changed.

Imposed pan conditions apparently cause less change in the loaf volume and other characteristics in loaves from low form pans. Thus we may have a partial explanation of why the low form pan tends to give less loaf volume variability than the tall pan in a set of replicate bakes. Doubtless there are differences not commonly detectable in the condition of different pans in a set, which are reflected less when the low form pan is used.

In summarizing the work of others and in the light of these studies, this committee would offer the following list of advantages and disadvantages for the tall and low form pans as recommended in the official A. A. C. C. Baking Test procedure.

Tall Form A. A. C. C. Baking Pan

Advantages

This pan is very desirable when the baking procedure is modified by proofing to a definite height or volume instead of for a definite time.

Much basic reference data have been accumulated by the use of this pan and photographs of exterior and interior types of loaves serve as valuable points of reference in reporting baking results.

Differentiation of flours with respect to break and shred is more pronounced using this pan. Good break and shred occurring only with the best flours in this respect.

A tall form pan subjects the flour to a severe test condition in the bake (Briggs, 1913; Lewis and Whitcomb, 1928).

Disadvantages

It is difficult to properly place doughs in the pan.

The dough appears to be cramped and is forced to give an elongated grain and possibly an exaggerated grain differentiation that is out of proportion to commercial significance, which in certain kinds of flour

testing may be considered an advantage. Upper grain may differ from that in the base of the loaf.

Loaves frequently tend to drag on one end of the pan in the oven-spring giving an erratic test loaf.

Differences in the condition of various pans in a set will reflect more on loaf volume variability and loaf type than when the low form pan is used.

If the use of a tall form pan is considered an undesirable manner to impose a severe test condition on the flour, then this pan is at a disadvantage. A severe pan condition and severe formula or other modification add complexity to an already complex problem.

Low Form A. A. C. C. Baking Pan

Advantages

Doughs are more easily placed in the pan.

The loaf produced has more nearly commercial loaf proportions and symmetry, and grain and texture, making scoring of the loaf a matter that requires less specialized experience and interpretation.

A wider range of loaf volumes is available for flour differentiation.

Disadvantages

It is difficult to modify the basic procedure to proof to a definite height as some doughs tend to spread and flatten out in later proofing stages.

Most loaves have even break and shred making shell top tendencies more difficult to detect and grain and shred differences harder to differentiate.

Frequently loaves exhibit a double break which if avoided should decrease loaf volume variability.

Suggestions for Selecting Suitable Baking Pans

When selecting the most suitable size and shape of baking pan for a definite quantity of dough in a standard baking test outline, these procedures might be followed:

The fundamental relation between pan size and shape should be studied in relation to dough expansion in the proof and in the oven which would possibly result in a different size pan for flours of different expansion potentialities for optimum conditions.

The whole range of pan sizes and shapes should be studied experimentally with a number of flours of average expansion potentialities to obtain the optimum adjustment for greatest accuracy and precision in loaf volume and loaf type.

A maximum number of qualified test-bake operators should be consulted through collaborative experiments with various types of flours and pans, and the size and shape of pan selected through the analysis of adequate data and arbitrary opinion as to what dimensions would make one pan more acceptable than another.

In a sectional meeting of the Pioneer Section discussing the symmetry of the loaf from the low form A. A. C. C. pan it was suggested that the pan be modified to have less pitch in the sides and to increase the size of the pan. Following these suggestions, pans were made up in which the top dimension was the same as the regular official low form pan, but the bottom dimensions were increased to give the sides nearly the same pitch as the tall form pan. This pan designated as Pioneer experimental pan No. 1 (2XX tin, top dimensions 11.5 cm. by 7.0 cm. —bottom dimensions 10.5 cm. by 6.0 cm.—depth 5.0 cm.) proved to be favorable for study. Preliminary studies included in Table I show that this modified low form pan gave a definite increase in loaf volume over the official low form pan and no significant difference in variability.

TABLE I

BAKING PAN DATA

Comparison of Loaf Volume and Variability
Proof time 55 minutes

	2XX tin Tall form pan			2XX tin Low form			2XX tin Modified low form (Pioneer No. 1 pan)		
	N	X cc.	C.V. %	N	X cc.	C.V. %	N	X cc.	C.V. %
Flour No. 1	16	672	2.02	16	678	1.44	15	726	2.02
Flour No. 2	16	589	1.51	16	615	1.74	16	626	2.05
Flour No. 3	16	626	2.02	15	650	1.60	16	667	1.46
Flour No. 4	15	658	2.16	15	667	2.10	15	683	2.08
Flour No. 5	16	558	1.60	16	587	2.16	16	596	1.84
Flour No. 6	16	683	3.43	16	722	2.97	16	728	3.31
Flour No. 7	16	575	1.72	16	591	1.54	16	604	1.37
Average		623	2.07		644	1.93		661	2.02

Summarizing the opinion of 16 collaborators who have used the Pioneer No. 1 pan, it compares with the official low form pan as follows:

Doughs were easier to pan and there was sufficient room in the bottom of the pan to allow the dough to be pulled to the side of the pan, thereby giving greater insurance of a one side break. Undeveloped doughs gave a characteristic double break.

Doughs did not proof so high above the pan, and had less opportunity to flatten out. This should improve the use of the low pan in proofing to a definite height or volume. The proof time was shorter.

Loaves had more nearly commercial loaf symmetry and proportion which was desirable in establishing the bake shop tendencies of a flour.

The larger loaf volume obtainable, possibly gives a greater range for flour differentiation.

The size of the pan was more nearly adjusted to the dough quantity and the A. A. C. C. molding procedure. It was suggested that the pan is still too small for the dough quantity.

Pioneer experimental pan No. 2 which was wider than the No. 1 pan gave loaves of less desirable appearance and no definite volume increase. It was hoped that this pan would give a loaf that could be cut crosswise, but apparently the A. A. C. C. molding procedure produces a grain structure unsatisfactory for this practice.

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